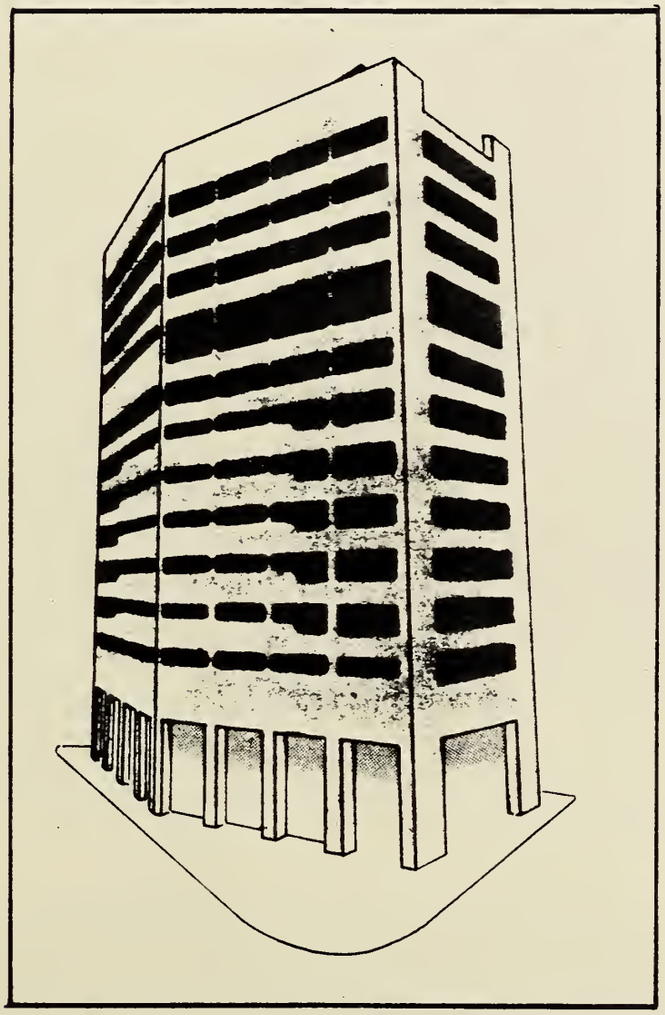
UNITED STATES DEPARTMENT OF AGRICULTURE

HUMAN NUTRITION RESEARCH CENTER ON AGING

TUFTS UNIVERSITY



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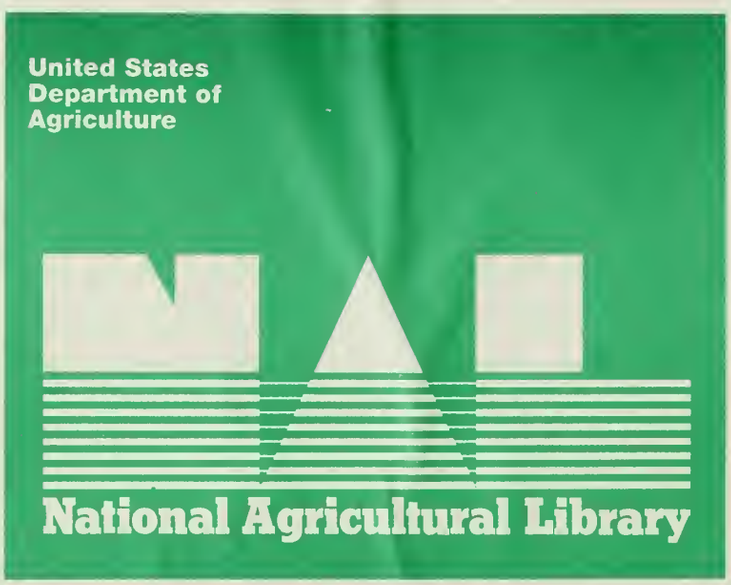
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INTRODUCTION

The USDA Human Nutrition Research Center on Aging was established by Congress through the Food and Agricultural Act of 1977. It is operated by Tufts University under the authority of the United States Department of Agriculture (USDA) as a government-owned, contractor-operated facility. The HNRC facility opened officially in November 1982.

The establishment of the HNRC was a major response of the Federal Government to the growing awareness of the need for better nutrition guidance for the American public throughout the life cycle. The overall mission of the HNRC is to explore the relationship between nutrition and good health and to determine the nutritional and dietary requirements of the maturing and elderly population. The interaction between nutrition and the onset and progression of aging is of special concern. HNRC scientists employ cell and molecular biology, animal models, human metabolic and field studies to better understand the processes of nutrient utilization and metabolism and thereby determine ways by which diet in combination with genetic and environmental factors may promote health and vigor over the life span.

Scientists at the HNRC are addressing three general questions of central importance to their mission:

What are the nutrient requirements necessary to obtain optimal function and well being for a maturing population?

How does nutrition influence the progressive loss of tissue function with aging?

What is the role of nutrition in the genesis of major chronic, degenerative conditions associated with the aging process?

This booklet describes the expertise of staff scientists, the four specialized core facilities and principal research programs at the HNRC. These programs are administratively described by the USDA through Current Research Information System (CRIS) units. These outlines are intended to highlight the scope of the programs and their recent achievements but do not provide a comprehensive definition of all aspects of HNRC research efforts. Those personnel and projects which are supported in part or in whole by non-USDA extramural support are marked with an asterisk.

HNRC Scientific Staff
Expertise Statements

Joseph Berger, PhD. (University of California). Scientist III. Biochemist developing lens cells in culture as a model in which to monitor and attenuate age-related decrements in protein metabolism. Expertise in cell culture, microinjection, protein degradation kinetics.

David Beguin, PhD (Washington State University). Research Associate. Nutritionist with experience in trace element nutrition. Currently working on zinc deficiency in animal models.

Jeffrey Blumberg, PhD (Vanderbilt University). Assistant Director/ Scientist I. Pharmacologist/Toxicologist with experience in preclinical behavioral and biochemical toxicological assessment. Current research interests in xenobiotic-nutrient interactions in the elderly and the role of dietary antioxidants during the aging process.

Kew M. Chee, PhD (Michigan State University). Visiting Scientist. Animal nutritionist with research interests in fatty acid requirements and fatty acid metabolism.

Michael Cohen, PhD (University of Texas). Research Associate. Biochemist with experience in cellular biology. Current research projects include examining the mechanisms of regulation of ion transport across intestinal cell membranes.

Jeffrey Cohn, PhD (Monash University). Research Associate. Nutritionist with research interests in cholesterol and lipoprotein metabolism in nutrition and atherosclerosis.

Gerard E. Dallal, PhD (Yale University). Scientist II. Statistician with interests in experimental design and observational studies. Active research areas include categorical data analysis, clustering, and statistical computing.

Bess Dawson-Hughes, MD (Tufts University). Assistant Medical Officer/ Scientist II. Physician trained and certified in Internal Medicine and Endocrinology. Laboratory experience in protein chemistry and cellular enzyme assays. Clinical research activity in metabolic bone disorders.

Johanna Dwyer, DSc (Harvard University). Scientist I. Nutritionist with expertise in diet and metabolism, specifically in obesity and energy balance, chronic degenerative conditions, and dietary methodology. Current research efforts in dietary recall methods, effects of diet on urine pH, and effects of diet on cariogenicity and oral health.

William Evans, PhD (Ball State University). Chief, Human Physiology Laboratory/Scientist II. Exercise physiologist studying muscle and exercise biochemistry in animals and man. Research interests in whole body protein dynamics using stable isotope infusion techniques, adaptation of muscle and whole body to exercise and aging and body composition.

David Fell, PhD (University of Wisconsin). Research Associate. Nutritionist with research interests in folate metabolism, folate-nutrient interactions and vitamin A.

Jacques Genest, MD (McGill University). Research Associate. Cardiologist with research interests in diet and apolipoprotein gene polymorphism in coronary artery disease.

Stanley N. Gershoff, PhD (University of Wisconsin). Senior Scientist. Nutritionist with experience in biochemical studies focused on vitamin metabolism and the metabolic relationships of nutrients in experimental animals and human subjects. Extensive experience in domestic and international public health nutrition.

Barbara Gilchrest, MD (Harvard University). Chief, Cutaneous Gerontology Laboratory/Senior Scientist. Physician board certified in Internal Medicine and Dermatology. Laboratory experience in cell biology. Current areas of research include influence of environmental factors on in vitro aging and growth control in cultured skin-derived cells. Clinical interests in skin aging and photomedicine.

Junxian Gong. BS (Wuhan Medical University). Visiting Scientist. Nutritionist with research interests in lipid metabolism and dietary linoleic acid requirements of the elderly.

Philip Gordon, PhD (University of Missouri). Scientist III. Nutritional biochemist with expertise in natural product isolation, trace element analysis, ligand metal interaction, platelet function assessment, and cell culture. Current interests involve trace element metabolism and function using diverse experimental systems.

Yacoob Haroon, PhD (University of London). Scientist III. Analytical biochemist with research interest in vitamin K metabolism and high performance liquid chromatrography.

Stuart C. Hartz, ScD (Harvard University). Chief, Program in Nutritional Epidemiology/Scientist I. Biostatistician/Epidemiologist with experience in clinical trial design and management and drug epidemiology investigating both drug efficacy and adverse reactions. Current research program includes nutritional status surveys of the elderly and role of nutrition in cataractogenesis.

Russ B. Hauser, MD (Albert Einstein University). Clinician trained in sports and physical medicine. Research interests in strength training and aerobic exercise effects on skeletal muscle and cardiac function in the elderly.

Marc Hellerstein, MD (Yale University). Scientist III. Physician trained in Internal Medicine and Endocrinology with experience in nutrition and diabetes. Research interests include plasma protein synthesis and hepatic carbohydrate metabolism.

Michael Holick, PhD, MD (University of Wisconsin). Chief, Vitamin D and Bone Metabolism Laboratory/Scientist I. Chemist/Biochemist with expertise in endocrinology and nutrition. Current research interests include role of sunlight exposure in maintaining vitamin D nutrition and the role that aging has on this process.

Marijke E. Holtrop, MD, PhD (State University of Leiden). Scientist I. Physician with extensive experience in bone histology and histomorphometry. Main research interest is in the role of vitamin D and other nutrients on the bone mineralization process.

Paul Jacques, MS (Harvard University). Research Associate. Epidemiologist with expertise in study design, implementation, and data analysis for epidemiologic, clinical and laboratory studies.

Jessica Jahngen, BS (Ohio Wesleyan University). Research Associate. Current interests in elucidating factors and processes which initiate and regulate protein degradation. Expertise in HPLC methods, protein purification, radionuclides, and proteolysis regulation.

Zohrab Kassarjian, MD (American University). Visiting Scientist. Physician trained and certified in Gastroenterology. Current research includes B-carotene absorption and metabolism as a function of aging and nutritional status.

Yang-Cha Lee (Kim), PhD. (Massachusetts Institute of Technology). Visiting Scientist. Biochemical nutritionist with expertise in the lipid metabolism of polyunsaturated fatty acids and the fat soluble vitamins, particular vitamins A and E.

Peter Libby, MD (University of California). Scientist I. Physician trained and certified in Internal Medicine and Cardiovascular Disease. Laboratory experience in cell biology and biochemistry. Current area of research in growth and metabolism of vascular smooth muscle and endothelium. Clinical interests in cardiovascular diseases associated with aging.

Ruth Lipman, PhD (Worcester Polytechnic Institute). Research Associate. Biochemist interested in relationships between aging and protein metabolism as affected by environment and nutrients. Extensive experience in the growth of cells in vitro (including clonal culture), chromosome banding, micromanipulation of individual cellular components, signals for cellular replication, and ion transport.

Robert B. McGandy, MD, (Cornell University). Senior Scientist. Physician trained in nutrition, preventive medicine and anatomical pathology. Major research interests in the interrelationship among nutrition, environmental factors, chronic disease and aging.

Claire Mansur, MD, (University of Minnesota). Research Associate. Physician board certified in Internal Medicine. Research interest in cell culture techniques applied to problems of nutrition and the skin during aging.

Carol Meredith, PhD (Massachusetts Institute of Technology). Scientist III. Nutritionist with expertise in protein nutrition and metabolism. Current research involves effects of age and physical training on protein requirements in both humans and animals.

Mohsen Meydani, DVM (Tehran University), PhD (Iowa State University). Scientist II. Veterinarian/Nutritionist with expertise in drug-nutrient interactions, nutritional toxicology and nutritional biochemistry. Current area of research interest in nutritional modification of lipid peroxidation and drug metabolism in animal models and humans.

Simin Meydani, DVM (Tehran University), PhD (Iowa State University). Scientist II. Veterinarian/Nutritionist with expertise in immunology and arachidonic acid metabolism. Current research includes effects of dietary antioxidants on immune response in animal models and humans.

Frank Morrow, PhD (University of Georgia). Research Associate/Manager, Nutrition Evaluation Laboratory. Nutritional Biochemist with expertise in protein purification and characterization by chromatographic and HPLC techniques. Research interests include long-term nutrition assessment testing in the elderly and investigation of intracellular long chain fatty acid metabolism related to prostaglandin status.

Hamish Munro, DSc, MD (Glasgow University). Senior Scientist. Physician with expertise in nutrition and biochemistry. Current research studies on protein and iron metabolism and peptide hormones in placental function. Current research on aging and genome control mechanisms for the iron-storage protein ferritin and peptide hromones.

Monica Peacocke, MD (McGill University). Research Associate. Physican board certified in Internal Medicine. Laboratory experience with polymorphic DNA problesm. Research interest in applying tools of molecular biology to nutrition problems associated with the aging skin.

Betzabe M. Praeger, PhD (University of Delaware). Scientist II. Cell Biologist with expertise in tissue culture techniques, electron microscopy, photomicrography, autoradiography, and cytochemistry. Current research studying the effect of UV light upon proliferation and response to mitogens of skin-derived cells from humans of different ages.

Frank Praeger, PhD (University of Delaware). Scientist III. Cell Biologist with expertise in tissue culture techniques. Major interest in the nutritional impact at the cellular level of various exogenous factors. Currently investigating the nutritional impact of calcium on cellular aging.

Rahul Ray, PhD (Washington State University). Scientist III. Chemist with experience in organo-boron chemistry. Research focus on photo-affinity labelling of the vitamin D hormone.

Judy D. Ribaya-Mercado, DSc (Harvard University). Scientist III. Nutritionist with interest in vitamin B_6 effects on carbohydrate and lipid metabolism, nutritional factors affecting the formation of urinary calcium oxalate stones and relationships among nutrition, aging, lens metabolism and cataract formation. Current research efforts on the effects of age, atrophic gastritis, and intestinal flora on human vitamin B_6 requirements.

Irwin H. Rosenberg, MD (Harvard Medical School). Director/Senior Scientist. Gastroenterologist with research interests in intestinal absorption of vitamins and minerals, nutrient bioavailability, breath tests for study of carbohydrate absorption and nutrient interactions.

Robert Russell, MD (Columbia University). Associate Director/Scientist I. Clinical Nutritionist certified in Gastroenterology and Internal Medicine. Current research interests include biochemical and functional testing in protein, zinc, folic acid and vitamin A malnutrition and alcoholism in animal models and humans.

James Sadowski, PhD (University of Wisconsin). Chief, Nutrition Evaluation Laboratory/Scientist II. Nutritional Biochemist experienced in fat-soluble vitamin and essential fatty acid metabolism and function, protein purification and characterization, cell culture, and catecholamine analysis/metabolism. Current research interests focus on the metabolism and function of vitamin K with age-related changes in requirements, metabolism and function.

Ernst Schaefer, MD (Mt. Sinai School of Medicine). Chief, Lipid Metabolism Laboratory/Scientist I. Physician trained and certified in Internal Medicine, Endocrinology and Metabolism. Laboratory experience in lipoprotein biochemistry and metabolism. Areas of research interest include age-related changes in dietary, hormonal, and genetic regulation of plasma lipoproteins, and the relationship of lipoproteins to premature atherosclerosis and longevity.

Mary Schaefer, PhD (City University of New York). Educational psychologist with expertise in statistics, data management/analysis and report writing.

Jacob Selhub, PhD (Case/Western Reserve). Scientist I. Biochemist with expertise in vitamin absorption and metabolism and vitamin binding protein, focused particularly on folate nutriture.

Allen Taylor, PhD (Rutgers University). Scientist I. Biochemist with interests in protein degradation and protein aging in the eye. Laboratory experience with NMR, IR, UV-VIS spectroscopy, X-ray diffraction, electron-microscopy of macromolecular assemblies, protein purification, eye lens histology, immunofluorescence, immunological techniques.

Joseph Tecce, PhD (Catholic University). Senior Scientist. Psychologist with expertise in electroencephalography, psychophysiology, psycho-pharmacology, and neuropsychology. Current research interests in the area of neuropsychopharmacological studies of sleep, Alzheimer's Disease and neuropsychological studies of nutrition and aging.

Gueng-Wen Tong, PhD (Rutgers University). Research Associate. Organic chemist with expertise in hydrogen transfer oxidation and reduction reactions. Extensive experience with instrumental analysis including HPLC, GC, MS, NMR, AA and x-ray diffraction analysis.

Ann Webb, PhD (Loughborough University). Research Associate. Biochemist with current research interests in the photobiology of vitamin D in skin.

Richard Wood, PhD (University of Connecticut). Scientist II. Nutritionist with experience in laboratory and human studies. Research interests directed to factors which influence mineral and trace mineral balance, excretion and requirements.

Mina Yaar, MD (Haddassah Medical School). Scientist II. Physician with expertise in dermatology, tissue culture, protein separation, immunoprecipitation, immunofluorescent microscopy and polyclonal antibodies production and purification. Current research efforts involve studies on zinc, interferon, and "senescence proteins".

Ganessa Yogeeswaran, PhD (University of Toronto). Scientist I. Biochemist with experience in role of cell surfaces, glycoproteins and glycolipids of biomembranes in cell growth control and tumor immunology. Current research interests include molecular immunology, cytoplasmic and cell surface biochemistry, and the role of vitamins A and E and steroids in differentiation and carcinogenesis.

METABOLIC RESEARCH UNIT

GENERAL CAPABILITIES

The Metabolic Research Unit (MRU) supports research protocols utilizing normal, healthy volunteers recruited from the metropolitan Boston area. Included in 55,000 square feet of MRU space are individual quarters for 26 resident volunteers, a complete metabolic kitchen serving both resident and free-living volunteers, dining rooms, medical examination rooms, a medical records library, and swimming pool, sauna and other recreational areas. The MRU maintains a full staff of volunteer recruiters, medical and dietetics personnel as well as allied services including medical records, social work and recreation therapy. The MRU staff also provides the same set of comprehensive services for free-living volunteers in HNRC protocols. The Physiology Laboratory, providing a full range of body composition and functional capacity tests, is located within the MRU.

Professional recruiters in the Department of Volunteer Services identify interested persons in the community and arrange for their prescreening evaluations prior to participation in a study. A number of recruitment tools are used including presentations to community groups, exhibits, brochures and radio and newspaper advertisements. A quarterly newsletter from the MRU keeps volunteers aware of current activities at the HNRC. A computerized Recruitment Tracking System developed by the HNRC helps the staff identify eligible candidates for new studies as well as assess the effectiveness of recruitment techniques.

Research nurses and paraprofessionals in the Department of Nursing are responsible for carrying out research protocols. Nurse Practitioners take medical histories, conduct physical examinations and research tests and provide medical care under the supervision of staff physicians. The staff is able to implement a wide variety of nutrition protocols including metabolic balance studies.

Dietitians and paraprofessionals in the Dietary Department are responsible for the interpretation, implementation and successful outcome of the dietary component of research protocols. The MRU kitchen is designed and equipped to support metabolic research studies requiring precise nutrient control. This is accomplished by weighing all food to the nearest gram, selecting a limited range of high quality, nutritious foods and following extremely precise preparation and serving procedures.

Routine blood work, urinalyses, electrocardiograms and X-rays are incorporated as part of the volunteer screening process. Twenty-four hour physician coverage of the MRU is provided for protection of resident volunteers.

Administrative Staff

Bess Dawson Hughes, MD Assistant Medical Director	Provides daily oversight and management of all MRU divisions. Responsible for medical coverage of resident volunteers.

Judith Frazier, RN/BA Director of Nursing	Plans and directs nursing functions and responsibilities. Serves as HNRC representative on the Tufts Human Investigational Review Committee.
Patricia Gerrity, BS/RD Director of Dietetics	Provides overall administrative and clinical direction for nutrition services including design of research diets and assessment tools.
Paula Murphy, MSW Director of Volunteer Services	Develops and directs all recruitment efforts including advertising, computerized recruitment tracking and assignment of protocol stipends.

Ellen Shafer, BA, MA, Administrative Coordinator
Judith King, Staff Assistant

Clinical Staff

Mary O'Sullivan, RN, Head Nurse
Carol Anderson, BSN/MS/FNP, Nurse Practitioner
Heidi Peters, BSN/MS/ANP, Nurse Practitioner
Janice Quigley, BSN/MS/ANP, Nurse Practitioner
Therese Mackin, RN, Staff Nurse
Susan McGurk, BS/RN, Staff Nurse
Jean Stains, RN/AD, Staff Nurse
Karen Ruais, AD/RN, Staff Nurse
Mary Beth Doherty, RN, Staff Nurse
Leah Jacoby, RN, Staff Nurse
Margaet Whelan, BS/RN, Staff Nurse
Patricia Rand, BSN, CCRN, Staff Nurse
Kimberlee Lane, RN/BS, Staff Nurse
Arlene Tenney, RN/BS, Admissions Coordinator
Lois Miller, MSW, Clinical Social Worker
Agatha Tong, B.S., Recreation Therapist
Connie Davis, Nsg Assist Certificate, Nursing Assistant
Marie Merchant, Nsg Assist Certificate, Nursing Assistant
Bennette Strange, Nsg Assist Certificate, Nursing Assistant
Merlene Clarke, Nsg Assist Certificate, Nursing Assistant
Linda Tryder, Cert. Bus. Comm., Secretary
Charlotte Earner, Secretary
Lucille Maher, Receptionist
Michele Badash, BS, Recruiter
Angela Walton, MEd, Recruiter
Geraldine Petrowski, RRA, Medical Records Coordinator
Elizabeth A. Simonetti, AA, Secretary

Helen Rasmussen, MS, RD, Research Dietitian
Nadine Sahyoung, MS, RD, Research Dietitian
Dorigen Kenney, MS, Administrative Dietitian
Trudy Hedrick, Secretary
Jennifer Carter, AA, ADM, Metabolic Diet Technician
Diane Paradis, Metabolic Diet Technician
Vickie Irwin, B.S., Metabolic Diet Technician
Mazie McIntosh, Food Production Technician
Luebertha Barnes, Metabolic Nutrition Assistant
Verona Bembridge, Metabolic Nutrition Assistant
Paul Fuss, BFA, Metabolic Nutrition Assistant
Karen Geller, BS, Metabolic Nutrition Assistant
Julie Giampietro, BA, Metabolic Nutrition Assistant
Jacqueline Moriarty, BS, Metabolic Nutrition Assistant
Marlaine Parker, Metabolic Nutrition Assistant
David Saia, Utilitity Food Service Worker
Albert Swinson, Utility Food Service Worker

NUTRITION EVALUATION LABORATORY

GENERAL CAPABILITIES

The Nutrition Evaluation Laboratory provides clinical and specialized biochemical analyses for human and animal research studies. The laboratory and its staff are organized into three main functional units: the Specimen Processing Unit, the Clinical Core Unit and the Esoteric Procedures Unit.

The Specimen Processing Unit (SPU) is responsible for receipt, processing, storage and workload tracking of blood, urine and fecal samples. SPU personnel are responsible for the preparation of protocol-specific urine and blood collection vessels for use by the MRU nursing staff and for logging test requests for a given subject into the NEL's laboratory management information system.

The Clinical Core Unit (CCU) is licensed by the Commonwealth of Massachusetts to provide clinical results for approximately 30 different procedures related to hematology, blood chemistries and urinalysis. Such tests are routinely performed on all subjects who are prospective volunteers for ongoing research protocols. The NEL participates in both the College of American Pathologists external proficiency survey as well as a monthly national quality control survey conducted by Fisher Scientific.

The Esoteric Procedures Unit (EPU) provides approximately sixty specialized laboratory procedures on a protocol-specific basis. The procedures provided by the EPU are evaluative in nature or generate protocol-specific data from subjects previously admitted to an ongoing study. Day-to-day quality control of the esoteric procedures is monitored by use of NBS Standard Reference Materials or by commercially available serum and urine quality control materials.

The NEL's laboratory information management system is a DECVAX based clinical laboratory software package which has been modified for use in the research environment. The system provides for long term storage and archiving of 30,000 specimen identifications and includes on-line quality control checking, workload reporting and direct transfer of test results from the Cobas centrifugal analyzer to the subject's medical record files. Study results can be provided to HNRC investigators in a variety of hard copy report formats or by direct electronic transfer into an RS1 table. Direct transfer of data from the clinical data package to RS1 allows investigators to readily perform statistical and graphical analyses of their ongoing studies without the need to manually re-enter numerical or textual results.

Investigators

James Sadowski, Ph.D. Chief/Scientist II	Provides scientific leadership in the Laboratory. Assists in the design and development of research protocols. Develops new methods for the assessment of nutritional status.
Frank Morrow, Ph.D. Research Associate/Manager	Provides managerial and organization leadership. Monitors and projects workloads, assists with protocol implementation, establishes and monitors quality control procedures. Develops new methods for the assessment of nutritional status.

Tehnical Support

Christine Burke, BS, ASCP, Medical Technologist
Betty Citron, BS, Research Assistant
Donna Elkerton, BS, ASCP, Medical Technologist
Neill Joy, Processing Technician
Marla McEwen, BS, ASCP, Medical Technologist
Gayle Perrone, B.S., Research Assistant
Phil Powell, Laboratory Systems Coordinator
Diane Rajchel, MS, ASCP, Medical Technologist
Doug Shepard, M.S. Research Assistant

DIVISION OF COMPARATIVE BIOLOGY AND MEDICINE

GENERAL CAPABILITIES

The Division of Comparative Biology and Medicine comprises the laboratory animal research facilitity and associated veterinary capabilities and services. The facility consists of a core unit of 28 barrier-type animal rooms contained on two secured levels. Satellite animal rooms are available for temporary housing on laboratory research floors. The total area of the facility is approximately 30,000 gross square feet and has the capacity to house over 15,000 rodents as well as other species such as rabbits and primates.

The core facility was designed for an all inclusive clean-dirty corridor system with anteroom extensions in approximately half of the rooms. A dedicated elevator provides an extension of the clean-dirty corridor system among the different levels where laboratory animals are housed. Strict protocol monitors and sensors have been established for the climate and environment including temperature and humidity control, air exchange (15 complete changes/hour/room of 100% fresh, non-recirculated air at a mass displacement, low velocity air flow), appropriate positive or negative pressure in animal rooms and adjacent corridors and duration and intensity of illumination. Environmental conditions are monitored on a twenty-four hour basis. All animal rooms are supplied with continuous flowing, minimal bioburden, deionized water which can be used selectively from a closed automatic watering loop. Ten animal rooms are designed to permit experimental work with biohazardous agents requiring Class Three Containment according to CDC standards.

The Division offers a wide range of assorted support services including diet preparation, veterinary nurse and technician support, surgery, necropsy, histology (including scanning electron microscopy) and "stat" hematology and clinical pathology. The unit possesses fully equipped surgery and necropsy suites and a specialized animal diet kitchen. Comprehensive veterinary diagnostic testing is provided by the Tufts University Veterinary Diagnostic Laboratories.

Administrative Staff

Donald Smith, MS, RLAT, Manager	Provides administrative and scientific direction to the division to ensure compliance with research protocols and government regulations. Serves as Chairman of the Animal Care and Use Committee and the liaison between the Consulting Veterinary and division staff.

Technical Support

Mary Ellenberger, DVM, Consulting Veterinarian
Kathleen Bertone, Program/Development Coordinator
Christine Resmini, AS, HLAT, Lab. Animal/Research Technician
Patricia Treglia, BS, HLAT, Lab. Animal/Research Tehnician
Teresa Haire, BS, HLAT, Assistant Lab. Animal/Research Technician
Raymond Lee, BS, Lab Animal Caretaker
Anthony Sealy, Lab Animal Caretaker
Ralston White, Lab. Animal Caretaker

DIVISION OF SCIENTIFIC COMPUTING

GENERAL CAPABILITIES

The Division of Scientific Computing Division maintains a state-of-the art central computing facility, offering timesharing services through use of a VAXCluster system with VAX-11/750 and VAX-11/780 CPUs running the VMS operating system. Online disk storage of approximately one gigabyte is available through 3 RA81 Winchester disk drives and 2 RA60 removable disk drives. These disks are completely shared by both CPUs via a HSC50 disk controller. A high speed, 1600/6250 bpi tape drive is available for backup and for import and export of data. Graphics terminals are made available to the HNRC community in a central terminal room and on each of the laboratory floors. These terminals are connected through Ethernet terminal servers located in satellite equipment rooms. Also provided are a Hewlett Packard 8 pen plotter and a Tektronix plotter. To aid in the integration of microcomputers and word processors, the Division provides IBM PC-compatible and Macintosh computers linked to the VAXCluster and interfaced DECmate word processors with compatible word processing software on the VAX.

The VAXCluster supports a large set of biomedical data analysis software, including SAS, SAS/Graph, SPSSX, SPSSGraphics, BMDP and RS/1. More specialized software is also provided, including CLINFO, a package designed for data management and analysis of clinical studies, and NONLIN84, a package for pharmacokinetic modelling. For development of special purpose software systems the VAXCluster has a set of programming languages (Fortran, C, Basic, PL/1) and data base management tools.

The Division of Scientific Computing provides two major data bases to assist in calculation of nutrient intakes. GRAND, a nutrient data base developed by the USDA HNRC at Grand Forks, is structured with a hierarchy of sources. Interactive coding and data entry software is provided, as are data coding and quality control services. DPIF (Drug Product Information File), a commercial database modified by the Division, is used to assist researchers in monitoring nutrient supplement and medication intake.

The Division of Scientific Computing offers a continuing series of education programs designed to make HNRC technical staff self-sufficient in computer usage and to provide the necessary skills that allow laboratories to take full advantage of the available computing power. For tasks beyond the capabilities of individual units, Scientific Computing provides customized systems analysis, programming and data entry services. The Division works in close collaboration with biostatisticians at the Center to develop or otherwise provide the necessary analytical tools.

Administrative Staff

Saul Tannenbaum, Manager	Provides leadership to the Division. Directs software development, computer operations, data base development, data operations and the design and delivery of computer users training and education.

Technical Staff

Daniel Abrams, BA, Senior Systems Programmer
Karen Hall, BA, Programmer/Analyst
Eric Slosser, BA, Programmer/Analyst
Lorraine Scura, Data Operations Coordinator
Dorothy Beaton, BS, Data Coordinator
Paula Hansbury, BS, Data Processor
Janice Maras, AS, Data Processor Assistant
Deborah Doyle, Staff Assistant

CALCIUM AND BONE METABOLISM LABORATORY

CRIS: Role of Nutritional and Other Factors in Preventing Age-Related Loss of Bone Density

Mission

To examine ways in which diet and nutritional status in combination with exercise and hormones (parathyroid hormone, vitamin D, and estrogen) influence age-related loss of bone density. To determine the extent to which increased calcium intake can mitigate bone loss and prevent the development of osteoporosis and spontaneous fractures in the elderly. To define the impact of calcium supplement use on mineral stores in humans.

This mission is pursued through clinical studies in which the effects of modifying the diet and/or activity level on calcium absorption and bone density are measured in healthy elderly volunteers. In addition, studies are conducted in the rat in which bone is analyzed directly using histomorphometric techniques. This dual approach is designed to increase our understanding of the relationship between diet and bone health.

Investigator

Bess Dawson-Hughes, MD Laboratory Chief/Scientist II Assistant Professor, Medicine	Provides leadership to the laboratory. Develops new approaches and methods to measure the relationship between nutrition and bone health.

Technical Support

Laura Sadowski, BS, Research Assistant
Clanton Shipp, BS, Laboratory Technician
Paul Berger, BS, Laboratory Technician
Kathy Nolan, BS, Diet Technician*
Jean Slosek, MS, Data Analyst*
Dibby Falconer, MS, Recruiter/Nutritionist
Heidi Peters, RN-C/ANP, Nurse Practitioner
Judith King, Staff Assistant

Current Projects

Long-term effects of high calcium intake on bone density and blood pressure in healthy elderly women.*

Effects of physical activity on net intestinal absorption and renal handling of calcium in healthy postmenopausal women.*

Effects of age and calcium intake on net intestinal calcium absorption in healthy postmenopausal women.*

Effects of chronic low grade vitamin A excess on calcium balance and bone histomorphometry in aging Sprague-Dawley rats.*

Effect of dietary fiber on the bioavailability of calcium in elderly people with gastric atrophy.

Role of calcium and physical activity in the prevention of age-related loss of bone density in healthy postmenopausal women.*

Effect of nutrition and glucocorticoids on bone density in children with inflammatory bowel disease.

Effect of calcium supplements on iron storage in healthy premenopausal women.

Recent Research Accomplishments

Long-term effects of high calcium intake on bone density and blood pressure in healthy elderly women. A placebo-controlled, double-blind calcium supplement field trial, in which 360 healthy postmenopausal women will be studied for 5 years is underway. Recruitment and screening are extensive since women with calcium intakes exceeding 650 mg daily and those taking estrogen are excluded. Analysis of the cross-sectional dual photon absorptiometry bone scan data from the first 150 women screened has revealed that there is no age-related decline in bone density of the spine or hip in postmenopausal women who exceed 115% of ideal body weight in contrast to normal-weight women. Pilot data indicate that those women with a calcium intake of less than 400 mg daily lose mineral from the spine at a significantly greater rate than do those whose intake exceeds 800 mg daily.

Effects of age and calcium intake on net intestinal calcium absorption in healthy postmenopausal women. Preliminary data in elderly subjects indicates there is a significant increase in fractional net calcium absorption over 1-4 weeks after acutely lowering calcium intake. Fractional net absorption then declines over the ensuing 4 weeks despite a continued low calcium diet. The mechanism of this loss of adaptation is unclear.

Selected Recent Publication

Dawson-Hughes, B., Seligson, F.H. and Hughes, V.A. Effects of Calcium Carbonate and Hydroxyapatite on Zinc and Iron Retention in Postmenopausal Women. Am J Clin Nutr 44:83-88, 1986.

Dawson-Hughes, B., Reichlin, S., Goldman J., Stern, D. Regulation of Growth Hormone and Somatomedin C Secretion in Postmenopausal Women. Effect of Physiological Estrogen Replacement. J. Clin Endocrinol Metab 63:424-432, 1986.

Dawson-Hughes, B. The Role of Nutrition and Exercise in the Prevention of Osteoporosis. Geriatrics (In Press), 1986.

Russell, R.M., Bowman, B., Dawson-Hughes, B. and Blumberg, J.B. Nutrition and Aging. In: Chandra, R.K., ed., Basic and Clinical Nutrition, Lange Medical Publications (In Press), 1985.

Dawson-Hughes, B., Jacques, P. and Shipp, C. Dietary Calcium Intake and Bone Loss from the Spine in Healthy Postmenopausal Women. Am J Clin Nutr, Submitted 1986.

Dawson-Hughes, B., Shipp, C., Sadowski, L. and Dallal, G. Bone Density of the Radius, Spine and Hip in Relatio to Percent of Ideal Body Weight in Postmenopausal Women, Calcified Tissue International, Submitted 1986.

Mazess, R.B., Barden, H.S., Ettinger, M., Johnston, C., Dawson-Hughes, B., Baran, D., Powell, M. and Notelovitz, M. Spine and Femur Density Using Duel-Photon Absorptiometry in Normal US White Women. Calcified Tissue International, Submitted 1986.

CARDIOVASCULAR RESEARCH LABORATORY

CRIS: Nutrition, Aging and Cardiovascular Cell Function

Mission

To study the interactions of cardiovascular cells with nutrients and factors influenced by diet. The focus of this research is on vascular degenerative processes such as arteriosclerosis and hypertension that occur with aging, that are partially dependent on diet, and that are the major causes of the morbidity and mortality in the aging population.

Investigators

Peter Libby, MD* Laboratory Chief/Scientist I Associate Professor, Medicine	Leads research effort, responsible for organization and direction of the laboratory. Trains associates in research methodology.
Stephen Warner, MBBCh, PhD* Research Associate	Collaborates on scientific affairs, performs and supervises experiments, teaches laboratory techniques to technical staff.

Technical Support

Maria Janicka, DVM, Research Assistant*
Cynthia Galin, BA, BS, Research Technician*
Gary Friedman, BA, Research Technician*
Leslie Gordon, BA, Research Technician
Joan Flaherty, Staff Assistant

Current Projects

Characterization of lipoprotein-mediated injury to human vascular wall cells in culture and the protective effects of dietary antioxidants.

Effect of native and oxidized lipoproteins on the expression of genes for Interleukin-1 and platelet-derived growth factor in human monocytic cells.

Effect of lipoproteins and immunoregulatory molecules on expression of potentially atherogenic functions of human endothelial and vascular smooth muscle cells.*

Interactions of human vascular endothelial cells with prosthetic graft materials in vitro: Activation of human monocytes and endothelial cells.*

Recent Research Accomplishments

Human vascular endothelial cells express genes for platelet-derived growth factor in a regulated manner. The c-sis proto-oncogene encodes one chain of platelet-derived growth factor (PDGF), a vascular smooth muscle cell mitogen. Cultured human saphenous vein endothelial cells (HSVEC) contained c-sis mRNA (= 4 kb) detected by northern hybridization. Lipopolysaccharide (LPS) increased the level of c-sis mRNA in HSVEC in a dose- and time-dependent fashion. Concentrations of LPS as low as 10 ng/ml increased c-sis mRNA in HSVEC. These results are consistent with the increased secretion of PDGF-like material from bovine aortic endothelial cells due to LPS reported by others. The effect of LPS on HSVEC was selective since it has little effect on beta-tubulin or von Willebrand factor mRNA levels, nor did LPS kill HSVEC as determined by phase contrast microscopy and DNA measurements. Thus, endothelial cells cultured from adult human vessels can express the c-sis proto-oncogene and LPS increases the level of this mRNA under conditions that do not produce lethal injury. Future studies of c-sis gene expression in endothelial cells by variables related to diet (e.g. lipoproteins) must control for LPS, a ubiquitous laboratory contaminant. Regulated c-sis expression by adult human endothelial cells supports the concept of a paracrine control loop for smooth muscle proliferation in adult human blood vessels.

Human vascular smooth muscle cell and endothelial cells express genes for the immunoregulatory and inflammatory mediator Interleukin-1 (IL-1) in an inducible manner. IL-1 can induce potentially pathogenic functions of vascular endothelial cells. This mediator was formally thought to be produced primarily by activated macrophages. Human vascular endothelial and smooth muscle cells have been found to be capable of secreting IL-1. Under usual culture conditions human vascular smooth muscle and endothelial cells contain little or no mRNA for IL-1 alpha or beta, the two major species secreted by activated human mononuclear phagocytes. However, when these cells are exposed to bacterial LPS or tumor necrosis factor they accumulate IL-1 beta mRNA content at concentrations commonly found in many laboratory reagents. Both of these cell types accumulate IL-1 alpha mRNA under superinduction conditions (in the presence of cycloheximide). The increased levels of IL-1 mRNA result in increased synthesis and secretion of biologically active IL-1 measured by thymocyte co-stimulation. These results indicate a new and unsuspected role for vascular wall cells in autocrine and paracrine regulation of functions related to vascular degeneration, since IL-1 induces endothelial cell procoagulant and leukocyte adhesion activity. The earliest morphologic change in blood vessels of animals rendered hypercholesterolemic by dietary manipulation is increased adhesion of mononuclear phagocytes to the endothelium. Stimulation of IL-1 production by vessel wall cells injured by dietary variables may help to initiate the chain of events that leads to vascular degeneration over time. Because vascular wall cells are exquisitely sensitive to endotoxin, future investigations of the effects of dietary variables will have to exclude meticulously contamination by this ubiquitous substance.

Selected Recent Publications

Libby, P., Ordovas, J.M., Birinyi, L.K., Auguer, K.R. and Dinarello, C.A. Inducible Interleukin-1 Gene Expression in Human Vascular Smooth Muscle Cells. J Clin Invest 78: December, 1986.

Libby, P., Ordovas, J.M., Auguer, K.R., Robbins, A.H., Birinyi, L.K. and Dinarello, C.A. Endotoxin and Tumor Necrosis Factor Induce Interleukin-1 Gene Expression in Adult Human Vascular Endothelial Cells. Am J Path 124:179-186, 1986.

Libby, P., Raines, E.W., Cullinane, P.M. and Ross, R. Analysis of the Mitogenic Effect of Fetuin Preparations in Arterial Smooth Muscle Cells. The Role of Contaminant Platelet-Derived Growth Factor. J Cell Physiol 125:357-366, 1985.

Libby, P., Miao, P., Ordovas, J.M. and Schaefer, E.J. Lipoproteins Increase Growth of Mitogen-Stimulated Arterial Smooth Muscle cells. J Cell Physiol 124:1-8, 1985.

Libby, P., Wyler, D.J., Janicka, M.W. and Dinarello, C.A. Differential Effects of Human Interleukin-1 on Growth of Human Fibroblasts and Vascular Smooth Muscle Cells. Arteriosclerosis 5:186-191, 1985.

Libby, P. Long-Term Culture of Contractile Mammalian Heart Cells in a Defined Medium That Limits Non-Muscle Proliferation. Journal of Molecular and Cellular Cardiology 16:803-811, 1984.

Libby, P., O'Brien, K.V. The Role of Protein Breakdown in Growth, Quiescence, and Starvation of Vascular Smooth Muscle Cells. J Cell Physiol 118:317-323, 1984.

Libby, P., O'Brien, K.V. Culture of Quiescent Vascular Smooth Muscle Cells in a Defined Serum-Free Medium. J Cell Physiol 115:217-223, 1983.

CUTANEOUS GERONTOLOGY LABORATORY

CRIS: Nutrition and Aging Changes in Skin-Derived Cells

Mission

To understand cutaneous aging and the impact of nutrient intake and other potentially modifiable environmental factors on the apparent chronologic aging process in human skin. To devise dietary or related strategies for reducing the negative impact of such factors on cutaneous appearance and function in the elderly.

The laboratory pursues this mission principally using cultured human skin-derived cells from young and old healthy volunteers. A major beneficial consequence of this work should be the establishment of systems permitting investigation of nutritional impacts on growth and differentiated function at the cellular level. To date, virtually all laboratory-based nutrition research in this area has been done on whole animals and thus could not address such questions directly.

Investigators

Barbara Gilchrest, MD Laboratory Chief/Senior Scientist Professor, Dermatology	Provides leadership to the laboratory. Develops skin-derived tissue culture systems and examines clinical relevance of the work.
Mina Yaar, MD* Scientist III Assistant Professor, Dermatology	Conducts research on control of growth and differentiation in the keratinocyte and melanocyte.
Frank Praeger, PhD Scientist III	Conducts research on the influence of calcium and donor age on proliferative rates, confluent density and differentiation of cultured human keratinocytes and fibroblasts.
Betzabe Praeger, PhD* Scientist II	Conducts research on the influence of donor age on the ability of human keratinocytes and fibroblasts in culture to respond to mitogens.
Phil Gordon, PhD* Scientist III	Conducts research on purification of keratinocyte and melanocyte growth promoting factors and the influence of retinoids on these cells in culture.
Monica Peacocke, MD* Research Associate	Conducts research on molecular research mechanisms of cell aging and retinoid/calcium effects on cultured keratinocytes, fibroblasts and melanocytes.
Claire Mansur, MD* Research Associate	Assists in ongoing laboratory research.

Technical Support

Michael Vrabel, BS, Research Assistant
Deborah Woodward, BS, Research Assistant*
Toby Kauffman, Staff Assistant

Current Projects

Purification and characterization of new growth factors for human keratinocytes and melanocytes from bovine and human hypothalamic extracts.

Effect of carotenoids on UV-induced aging in human skin cells.

Effect of donor age and nutrient milieu on human skin cell production of and responsiveness to autocrine growth factors.

Effect of donor age, divalent metals and nutrient milieu on growth and maturation of cultured human skin cells.

Influence of retinoids on growth and UV-stimulated pigment production in cultured human melanocytes.*

Effect of donor age and prior sun exposure on DNA repair capacity of cultured human skin cells.*

Physiologic role of interferon in epidermal growth and differentiation.*

Recent Research Accomplishments

Development of a system for cultivation of normal human epidermal melanocytes and nevus cells. Pigment cells are influenced by nutrient intake, as reflected by pigment dilution in protein deficiency states and diffuse hyperpigmentation in niacin deficiency. A system for their in vitro cultivation have been developed. The cultured cells can be serially passaged, maintain their in vivo structural characteristics and continue to produce melanin pigment. Studies demonstrate marked substrate responsiveness of melanocytes and striking sensitivity to media components including retinoids. A method for physiologic UV-irradiation has been developed and demonstrates a direct effect of UV on melanogenesis.

Further purification of melanocyte growth factor (MGF) and keratinocyte growth factor (KGF) from hypothalamus. Improved extraction procedures have provided high yields and should facilitate the purification of these novel growth factors. Sequential column chromotography and HPLC have yielded an apparently pure peak with KGF biologic activity that is being characterized by mass spectroscopy. MGF biologic activity similarly co-elutes with single O.D. peak resulting from a second purification scheme.

Influence of calcium ion concentration on proliferation of human fibroblasts. Elevation of calcium concentration in basal nutrient medium has been found to greatly increase confluent density of newborn dermal fibroblasts. This increase is maintained through many passages in culture and is independent of fetal bovine serum concentration or cell growth rate. The effect is dependent on donor age: a doubling of calcium concentration increases cell yield approximately 350% for newborns, 150% for young adults and 20% for old adults. Thus, elevated calcium concentration decreases density-dependent growth inhibition of fibroblasts in vitro and this effect is donor age dependent. Elevated calcium appears toenhance fibroblast responsiveness to serum mitogens and/or mimic membrane effects of other growth regulatory molecules.

Influence of calcium and strontium on cultured keratinocytes. Calcium is widely recognized to promote terminal differentiation of human keratinocytes. To further explore the mechanism(s) of this effect, cells were grown in varying concentrations of calcium and the similar divalent cation strontium. Strontium was found to be highly growth stimulatory for keratinocytes but failed to induce terminal differentiation at any concentration. These data suggest that calcium has direct effects on both proliferation and differentiation but that stronium is capable of substituting only in the former pathway. The observation of keratinocyte growth stimulation raises the question of a physiologic role for stronium in the epidermis.

Human interferon profoundly and reversibly inhibits cultured keratinocyte growth. Interferons (IFN) are known for their antiviral activity that have subsequently been shown to inhibit the growth of many transformed and normal cell types. IFN was produced by keratinocytes in response to viral infection and cloned human alpha-IFN or beta-IFN profoundly and reversibly inhibited keratinocyte growth and promoted terminal differentiation. In the absence of viral infection, both in vivo and in vitro actively proliferating keratinocytes bound anti-IFN antibodies to fixed tissue sections or cultures and to extracts of cell proteins; extracts of the cultured cells contained small amounts of anti-viral activity. These data suggest IFN may function as a physiologic negative growth factor or chalone of epidermal growth.

Cultured human skin-derived cells show an age-related loss of growth factor responsiveness. Keratinocytes and fibroblasts derived from newborn foreskin respond significantly better to mitogens in short term culture than do the same cells derived from adult donors. This age-associated change was also found using cells from young adult vs. old adult donors. Results are consistent with the hypothesis that impaired wound healing and immuno-surveillance in the elderly may be attributable to specific biochemical changes at the cellular level.

Selected Recent Publications

Gilchrest, B.A., Albert, L.S., Karassik, R.L. and Yaar, M. Substrate Influences Human Epidermal Melanocyte Attachment and Spreading in Vitro. In Vitro 21:114-120, 1985.

Yaar, M., Karassik, R.L., Schnipper, L.E., Szabo, G., Gilchrest, B.A. Effects of Alpha and Beta Interferons on Cultured Human Keratinocytes. J Invest Dermatol 85:70-74, 1985.

Praeger, B.M. and Gilchrest, B.A. Growth Factor Responsiveness Declines During Adulthood for Human Skin-Derived Cells. Mech Ageing Devel 35:185-198, 1986.

Yaar, M., Palleroni, A.V. and Gilchrest, B.A. Normal Human Epidermis Contains an Interferon-Like Protein. J Cell Biol (In Press).

Praeger, F.C. and Gilchrest B.A. Influence of Increased Extracellular Calcium Concentration and Donor Age on Density Dependent Growth Inhibition of Human Fibroblasts. Proc Soc Ex Biol Med 182:315-321, 1986.

Gordon, P. R., Treloar, V.D., Vrabel, M.A. and Gilchrest B.A. Relative Responsiveness of Cultured Human Epidermal Melanocytes and Melanoma Cells to be Selected Mitogens. J Invest Dermatol (In Press).

Gilchrest, B.A. Defined Culture Systems for Pharmacologic and Toxicologic Studies. Br J Dermatol 115:17-23, 1986.

Gilchrest, B.A. and Gordon, P.R. Impact of Aging and Nutrition on Human Skin: Studies at the Celular Level. In: Nutrition and Aging. SN Gershoff, M Hutchinson, HN Munro (eds). Academic Press: Orlando FL, pp. 35-43, 1986.

GASTROINTESTINAL/MICRONUTRIENT NUTRITION LABORATORY

CRIS: Micronutrient Requirements of the Elderly
Macronutrient Requirements of the Elderly
Bioavailability of Nutrients in the Elderly

Mission

To determine how aging and associated factors such as medication use affect the absorption and metabolism of vitamins and minerals and to study the bioavailability of micronutrients during the aging process. Results from this work will indicate if changes to the recommended dietary allowances of vitamins and minerals are needed for the elderly.

This laboratory pursues its mission using free-living and resident human volunteers and experimental animal models. Elderly subjects with atrophic gastritis or hypochlorhydria are frequently employed in bioavailability studies as they represent a significant subpopulation at risk of impaired nutrient absorption. Perfused intestinal segments and mesenteric lymph cannulae are employed in animal models characterizing the kinetics, energy requirements and age-associated changes in fat soluble vitamin uptake and clearance.

Investigators

Robert Russell, MD Laboratory Chief/Scientist I Associate Professor, Medicine	Provides overall leadership to the laboratory. Conducts studies on absorption of folate and vitamin A in elderly humans with and without gastric atrophy and the effect of bacterial overgrowth on nutrient bioavailability. Also, studies the intermediary metabolism of vitamin A as affected by aging: the transfer characteristics of vitamin A across the unstirred water layer intestinal membrane and the transfer of retinyl esters between various lipoprotein fractions in vivo.
Judy Ribaya-Mercado, DSc Scientist III	Studies the effect of aging on vitamin B6 bioavailability and the enterohepatic circulation of vitamin A.
Stanley Gershoff, PhD Senior Scientist Professor/Dean, Nutrition	Collaborates on studies of micronutrient requirements, particularly vitamin B6.
Zorhab Kassarjian, MD Visiting Scientist	Conducts research on beta-carotene absorption/metabolism as a function of aging and lipid ingestion. Investigates factors affecting calcium bioavailability in atrophic gastritis.

Yang-Cha Lee, MD Visiting Scientist	Conducts research on the absorption and enterohepatic circulation of vitamin E using animal models.
Gueng-Wen Tong, PhD Research Associate	Conducts studies on polar metabolites of vitamin A and the effects of UV radiation on skin retinoid metabolism.

Technical Staff

Elizabeth Phelan, BA, Research Technician
Barbara Golner, Research Technician
Stephen Krasinski, MS, Graduate Research Assistant
Sophia Holmgren, Research Technician
Cindy Fulton, Secretary

Current Projects

Development of animal models to study the enterohepatic circulation of vitamins A and E.*

Determination of the vitamin B6 requirements of elderly people.

Effect of atrophic gastritis on the synthesis of vitamin B6 by intestinal flora in elderly people.

Influence of light, antioxidants and aging on retinal biochemistry and function in Long-Evans rats.

The susceptibility to vitamin A deficiency of the aging Sprague Dawley rat.

Effects of chronic, low-grade vitamin A toxicity on bone health in aged Sprague Dawley rats.

Effect of dietary and supplemental vitamins A and E on serum retinol, retinyl ester and alpha-tocopherol levels in the elderly.

Effect of age, hyperlipidemia and malabsorption on retinyl ester tolerance curves in humans.

Effects of age and Apo E isoforms on chylomicron-retinyl ester plasma clearance in humans.*

Effect of fiber on the bioavailability of calcium in normal and achlorhydric subjects.

Effects of aging on the absorption and metabolism of beta-carotene in guinea pigs.

Post-prandial alterations of plasma lipids and retinyl esters in Sprague Dawley rats and humans.*

Effect of mild to moderate atrophic gastritis on absorption of protein-bound and crystalline vitamin B12 in the elderly.

Effect of antacids or H2 blockers on folate absorption in the elderly.

Effect of carotene feeding on growth and tissue/blood carotenoid and retinoid contents.

Age and energy requirements for vitamin A intestinal epithelial cell uptake and effects of aging on characteristics of the unstirred water layer.

Recent Research Accomplishments

The effect of protein malnutrition on weanling or adult rat retina. The adult rat retina is resistant to the effects of low dietary protein. However, low-protein diets in young growing rats cause functional retinal abnormalities and depression of plasma albumin, protein and taurine compared to control animals. Although plasma retinol is lowered by protein malnutrition, this does not result in depletion of ocular vitamin A stores. Therefore, protein malnutrition has direct detrimental effects on vitamin A nutritional status. The abnormal retinal function is reversible after unrestricted access to a normal diet.

Taurine supplementation and retinal function of protein-malnourished rats. Weanling rats fed a low-protein, taurine-free diet develop abnormal retinal function that may be due to systemic taurine deficiency. Supplementation with taurine causes a normalization of circulating and tissue taurine stores. However, tests of retinal function become worse indicating a toxic effect due to taurine feeding. The retinopathic effects of feeding physiologic levels of taurine to low-protein fed rats are not related to classical symptoms of amino acid toxicity because taurine supplementation did not cause further growth impairment or decreased food intake. Thus, retinal function testing may be a sensitive indicator of amino acid toxicity and retinal taurine depletion during low-protein feeding is the result of retinal damage rather than a cause of retinal tissue loss.

Prevalence and severity of atrophic gastritis in an elderly population: effect on serum nutritional indicators. Atrophic gastritis of the fundic gland mucosa increases with age and is associated with decreased hydrochloric acid secretion. Severe atrophic gastritis may lead to vitamin B12 deficiency and pernicious anemia. In an elderly Boston population the prevalence rate of atrophic gastritis (determined by the pepsinogen I/pepsinogen II ratio in the blood) was 18% (8% with severe cases). The mean serum vitamin B12 level was significantly lower in atrophic gastritis although other nutritional indicators, e.g. serum iron, folic acid, vitamins A and E, were unaffected.

Effect of gastric atrophy on nutrient bioavailability. Severe atrophic gastritis may result in impaired digestion. However, even mild degrees of atrophic gastritis appear to impair the absorption of protein-bound vitamin B12. No impairment of crystalline vitamin B12 absorption due to atrophic gastritis was found. Folic acid was also found to be malabsorbed in elderly subjects with gastric atrophy and achlorhydria. Folic acid absorption was decreased in normal volunteers when the folate was administered along with an antacid or an H2 blocking agent. Serum folate levels were within normal ranges in the atrophic gastritis subjects although their acidity and bacterial counts were higher in the proximal small intestine than controls. Bacteria cultured from aspirates of atrophic gastritis subjects were able to synthesize folates. Thus, atrophic gastritis results in folate malabsorption but not in folate deficiency possibly due to increased bacterial synthesis of folate in the small intestine.

Age-related changes in vitamin A tolerance curves. Despite low dietary vitamin A intakes, vitamin A deficiency in the elderly is rare and vitamin A levels are maintained throughout life. In rats, uptake of vitamin A from perfused intestinal segments increased in an age-related manner. In humans, vitamin A tolerance curves were constructed by plotting the retinyl ester concentration against time after oral vitamin A administration. Mean peak height and area under vitamin A tolerance curves were greater in the elderly than in young adults. These results could be due to an age-related increase in vitamin A absorption and/or a decrease in hepatic retinyl-ester clearance. These questions are now being investigated.

Selected Recent Publication

Bankson, D.D. and Russell, R.M. Determination of Retinyl Esters and Retinol in Serum or Plasma by Normal-Phase High-Performance Liquid Chromatography (HPLC): Method and Applications. Clin Chem 32,1:35-41, 1986.

Vincent, M.L., Russell, R.M. and Sasak, W. Folic Acid Uptake Characteristics of a Human Colon Carcinoma Cell Line, Caco-2: A Newly-Described Cellular Model for Small Intestinal Epithelium. Human Nutr:Clin Nutr 39C:355-360, 1985.

Krasinski, S.D., Russell, R.M., Furie, B.C., Furie, B., Kruger, S. and Jacques, P. Subclinical Vitamin K Deficiency in Patients with Inflammatory Bowel Disease and/or Malabsorption Disorders. Am J Clin Nutr 41:639-643, 1985.

Garrett-Laster, M.S., Russell, R.M. and Jacques, P. Impaired Taste and Olfaction in Patients with Liver Disease: The Role of Vitamin A. J Human Nutr 38(2):161-240, 1984.

Russell, R.M., Krasinski, S.D., Samloff, I.M., Jacob, R.A., Hartz, S.C. and Brovender, S.R. Folic Acid Malabsorption in Atrophic Gastritis: Compensation by Bacterial Folate Synthesis. Gastroenterology (In Press).

Krasinski, S.D., Russell, R.M., Samloff, I.M., Jacob, R.A., Dallas, G.E., McGandy, R.B. and Hartz, S.C. Fundic Atrophic Gastritis in an Elderly Population: Effect on Hemoglobin and Several Serum Nutritional Indicators. J Am Ger Soc (In Press).

Bankson, D.D. and Russell, R.M. Protein Energy Malnutrition and Taurine Supplementation: Effects on Vitamin A Nutritional Status and Retinal Function in Rats. J. Nutr (In Press).

LIPID METABOLISM LABORATORY

CRIS: Lipoproteins, Nutrition, and Aging

Mission

To define the interrelationships between lipoprotein metabolism, nutrition and the aging process with particular emphasis on optimal diets in terms of fat and cholesterol content in the elderly to minimize cardiovascular risk factors and atherosclerosis. Studies are being carried out which: 1) establish biochemical parameters identifying individuals at risk of premature coronary artery disease (CAD) and optimal diets which minimize associated plasma lipoprotein abnormalities; 2) define short and long term regulation of plasma lipoproteins by diet; 3) define nutritional regulation of lipoprotein synthesis in vitro, and 4) define nutritional requirements for essential fatty acids in the elderly.

Methodologies established in the laboratory include: lipoprotein isolation by ultracentrifugation, automated enzymatic lipid analysis, gradient gel electrophoretic analysis of plasma lipoproteins, apolipoprotein isoelectric focusing, apolipoprotein quantitation by enzyme linked immunoassay, isolation of apolipoproteins by HPLC and standard column chromatography, fatty acid analysis by gas liquid chromatography, Hep G-2 and Caco2 cell cultures, DNA isolation and genomic blotting analysis, specific mRNA quantitation, and gene cloning and sequencing.

Investigators

Ernst Schaefer, MD* Laboratory Chief/Scientist I Associate Professor, Medicine and Nutrition	Provides leadership to the laboratory. Carries out nutritional and clinical studies, directs lipid and apolipoprotein analyses.
Jose Ordovas, PhD* Scientist III	Conducts molecular biology and tissue culture studies, carries out gene analysis and examines apolipoprotein synthesis in vitro.
Stefania Fava, MD Research Associate	Studies nutritional regulation of hepatic apolipoprotein synthesis in vitro.
Jeffrey Cohn, PhD* Research Associate	Studies nutritional regulation of apolipoprotein synthesis in vivo using stable isotopes.
Jacques Genest, MD* Research Associate	Studies apolipoprotein B gene polymorphism and atherosclerosis.
Mary Schaefer, PhD* Research Associate	Carries out statistical analysis, data management.

Kew Chee, PhD* Visting Scientist	Studies nutritional fatty acid requirements.
Jun Xian Gong, MD* Visting Scientist	Studies nutritional fatty acid requirements.

Technical Support

Judith McNamara, BS, MT, Research Assistant
Ann LaFleur, BS, Laboratory Technician
Natalie Coleman, BS, Research Technician*
Susan Cohn, BS, MS, Research Technician*
Alison Robbins, BS, Research Assistant*
Steven Johnson, BS, Research Technician
Jean-Eric Triau, MS, Graduate Research Assistant
Thomas Hughes, MS, Graduate Research Assistant
Lori Hennessy, MS, Graduate Research Assistant
Deirdre Rees, MS, Graduate Research Assistant
Hannia Campos, MS, Graduate Research Assistant
Patricia Wedge, Staff Assistant

Current Projects

Plasma lipoprotein and apoliporotein abnormalities and gene polymorphism in subjects with premature coronary artery disease and atherosclerosis.*

Effect of nutritional and hormonal alterations on low density lipoprotein subfractions.

Normal ranges, nutritional regulation and effect of genetic ApoE isoforms on plasma lipoproteins and apolipoproteins from the Framingham Offspring Study.*

Postprandial alterations in lipoproteins, apolipoproteins and fat-soluble vitamins in humans.

Effect of feeding and fasting on apolipoprotein synthesis in men from different age groups using stable isotope methodology.

Effects of age and apolipoprotein E isoforms on chylomicron-retinyl ester clearance.

Effect of estrogen on nutritional regulation of plasma lipoproteins in post-menopausal women.

The effect of cholesterol and saturated fat on lipoprotein kinetics and apolipoprotein hepatic and intestinal mRNA levels in Cebus monkeys.

Nutritional regulation of hepatic lipoprotein and apolipoprotein synthesis <u>in vitro</u> in Hep G-2 and Caco2 cells.

Recent Research Accomplishments

High density lipoprotein (HDL) deficiency is the single most common plasma lipoprotein abnormality observed in patients with CAD and is associated with a specific apoA-I gene polymorphism. An HDL cholesterol below the tenth percentile of normal was observed in 58% of patients with premature CAD. ApoA-I is the major protein of HDL. A specific apoA-I gene polymorphism was found in 4% of normals, 32% of CAD patients and 66% of patients with genetic HDL deficiency. This polymorphism is the first reported common gene marker for premature CAD. This marker may be useful in identifying subjects at increased risk for CAD and recommending prudent diets and other appropriate risk factor modifications. A new kindred with premature CAD, apoA-I and apoC-III deficiency has also been described. In addition, the presence of low molecular weight low density lipoproteins (LDL) in plasma which may enter the arterial wall more readily than large LDL has been found to be associated with male sex, aging, hypertriglyceridemia, HDL deficiency and premature CAD.

Normal ranges for plasma apolipoproteins (apo)A-I and B have been developed. ApoA-I and apoB plasma levels have been reported to be better predictors of premature CAD than plasma HDL cholesterol and LDL cholesterol concentrations, respectively. The first large, normal ranges for these plasma proteins have been developed based on the Framingham Offspring Study of 3500 people. These data indicate that: there are significant age-related increases in apoB, females have significantly higher apoA-I levels and lower apoB levels than do males, and decreased apoA-I levels and increased apoB levels are associated with the presence of CAD.

Apolipoprotein E isoform phenotypes are important determinants of total cholesterol and LDL cholesterol in the general population. Three different genetic forms of ApoE can be detected by isoelectric focusing of very low density lipoproteins. The common form of apoE is known as apoE3 while variant forms are apoE4 and apoE2. Subjects with the apoE4 allele have the highest LDL cholesterol levels while subjects with the apoE2 allele have the lowest. In contrast, apoE2/2 homozygotes are at increased risk for developing type III hyperlipoproteinemia.

Healthy octogenarians have normal lipoproteins. In a study of 160 healthy octogenarians free of CAD from the Framingham Heart Study there was no increased prevalence of subjects with low LDL cholesterol or high HDL cholesterol. HDL deficiency was extremely rate. Most subjects in the study were non-smokers.

Estrogens are effective in lowering LDL and raising HDL in postmenopausal women. Estrogen replacement in postmenopausal women has been shown to be extremely effective in lowering LDL cholesterol and raising HDL cholesterol in plasma.

Elderly subjects catabolize chylomicron triglyceride and retinyl ester constituents more slowly than younger subjects. Studies have been carried in young and elderly subjects given a fat-rich meal. Many subjects displayed two peaks of post-prandial hypertriglyceridemia. Elderly subjects had a delayed clearance of chylomicron triglyceride and retinyl ester. These findings have been verified with infusion studies.

Detection of essential fatty acid defiency based on the ratio of trienoic to tetraenoic acid. The normal trienoic: tetraenoic acid (Mead acid:arachidonic acid) ratio was found to be a 0.012 ± 0.004 in normal subjects. Increased amounts of Mead acid are produced in essential fatty acid deficiency resulting in an elevated ratio which was common in patients with various gastrointestinal disorders. Elevated ratios were also associated with hypertension, hypertriglyceridemia and HDL cholesterol deficiency.

Selected Recent Publications

Schaefer, E.J., Rees, D.G. and Siguel, E.N. Nutrition, Lipoproteins, and Atherosclerosis. Clinical Nutrition 5:99-111, 1986.

Ordovas, J.M., Schaefer, E.J., Salem, D., Ward, R.H., Glueck, C.J., Vergani, C., Wilson, P.W.F. and Karathansis, S.K. Apolipoprotein A-I Gene Polymorphism Associated with Premature Coronary Artery Disease and Familial Hypoalphalipoproteinemia. New England Journal of Medicine 314:671-677, 1986.

Siguel, E.N., Blumberg, J.B. and Caesar, J. Monitoring the Optimal Infusion of Intravenous Lipids. Arch Pathol Lab Med 110:792-797, 1986.

Triau, J.E., Arbetter, J. and Schaefer, E.J. Impaired Hepatocyte Binding, Uptake and Degradation of Glucosylated Low-Density Lipoproteins. Biochimica et Biophysica Acta 877:359-365, 1986

Schaefer, E.J. and Levy, R.I. Pathogenesis and Management of Lipoprotein Disorders. The New England Journal of Medicine 312: 1300-1310, 1985.

Libby, P., Miao, P., Ordovas, J.M. and Schaefer, E.J. Lipoproteins Increase Growth of Mitogen-Stimulated Arterial Smooth Muscle Cells. Journal of Cellular Physiology 124:1-8, 1985.

Schaefer, E.J., Ordovas, J.M., Law, S.W., Ghiselli, G., Kashyap, M.L., Srivastava, L.S., Heaton, W.H., Albers, J.J., Connor, W.E., Lindgren, F.T., Lemeshev, Y., Segrest, J.P. and Brewer, H.B. Familial Apolipoprotein A-I and C-III Deficiency, Variant II. Journal of Lipid Research 26:1089-1101, 1985.

Zannis, V.I., Ordovas, J.M., Cladaras, C., Cole F.S., Forbes, G. and Schaefer, E.J. mRNA and Apolipoprotein E Synthesis Abnormalties in Peripheral Blood Monocyte Macrophages in Familial Apolipoprotein E Deficiency. The Journal of Biological Chemistry 260:12891-12894, 1985.

NUTRIENT BIOAVAILABILITY LABORATORY

CRIS: Bioavailability of Nutrients in the Elderly

Mission

To examine the basis for the absorption, utilization and excretion of nutrients in maturing and elderly populations and to determine the interactions between foods and drugs relevant to nutrient requirements.

This laboratory pursues its mission using cell culture, subcellular membrane fractions, animal models and metabolic studies in humans. Research is focused on the mechanisms of absorption and excretion of water soluble vitamins such as folic acid and the effects of other dietary constituents on mineral balance.

Investigators

Irwin Rosenberg, MD Laboratory Chief/Senior Scientist Professor, Medicine and Nutrition	Provides overall direction to the laboratory research program.
Jacob Selhub, PhD* Scientist I Associate Professor, Nutrition	Directs studies on bioavailability and excretion of folate.
Richard Wood, PhD* Scientist II Assistant Professor, Nutrition	Directs studies on absorption and excretion of minerals and trace elements.
David Fell, PhD* Research Associate	Examines new approaches to folate analysis and effects of other nutrients on folate metabolism.
David Beguin, PhD Research Associate	Conducts research into dietary trace element requirements throughout the life span.

Technical Support

Cynthia Theall, MS, Research Assistant
Anna Giuliano, MS, Graduate Research Assistant
William Dutczak, Research Technician
Allison Harbour, Research Technician
Edythe Scott, Secretary

Current Projects

The physiology of digestion and absorption of polyglutamyl folate.*

Mechanisms of folate transport by the intestinal brush border.*

The effect of pH and drugs on folate absorption.

Membrane proteins and folate translocation.

The isolation and function of folate binding proteins.

The development of new techniques for tissue folate analysis.*

The effects of methionine on folate metabolism and DNA synthesis.

The effect of macronutrients on calcium and mineral absorption, excretion and balance.

The mechanisms of interaction between carbohydrates and minerals in the diet.

The regulation of mineral transport in CaCo 2 cells.

The mechanisms of the age-related decline in intestinal calcium absorption.*

Recent Research Accomplishments

This is a new program initiated October 1986.

Selected Recent Publications

Bengoa, J.M. and Wood, R.J. Magnesium. In: Absorption and Malabsorption of Mineral Nutrients. N. W. Solomons and I.H. Rosenberg, Eds., Alan R. Liss, Co., New York, 1984.

Zheng, J.-J., Wood, R.J. and Rosenberg, I.H. Enhancement of calcium absorption in rats by coadministration of glucose polymer. Am J Clin Nutr 41:243-5, 1985.

Bei, L., Wood, R.J. and Rosenberg, I.H. Glucose polymer increases jejunal calcium, magnesium and zinc absorption in humans. Am J Clin Nutr 44:244-7, 1986.

Zimmerman, J., Selhub, J. and Rosenberg, I.H. The role of sodium ion in the transport of folic acid in the small intestine. Am J Physiol 251:G218-G222, 1986.

Zimmerman, J,, Selhub, J. and Rosenberg, I.H. Trimethoprim and pyrimethamine, dihydrofolate reductase inhibitors are competitive inhibitors are competitive inhibitors of folate absorption. Am J Clin Nutr (In Press).

Zimmerman, J., Selhub, J. and Rosenberg, I.H. Competitive inhibition of folic acid absorption in rat jejunum by triamterene. J Lab Clin Med (In Press).

Rosenberg, I.H., Zimmerman, J. and Selhub, J. Analysis of the pH and Na+ Dependence of Intestinal Folate Transport. Proceeding of the 8th International Symposium on Pteridines and Folic Acid Derivatives, 1986.

Funfar, J., Wood, R., Carroll, K. and Rosenberg, I. Effect of age on 1,25(OH)$_2$ vitamin D stimulated calcium uptake in isolated rat enterocytes. Fed Proc 45:830, 1986.

Bowman, B.B., Selhub, J. and Rosenberg, I.H. Intestinal absorption of biotin in the rat. J Nutr 116:1266-1276, 1986.

NUTRITION AND CATARACT RESEARCH LABORATORY

CRIS: Effect of Nutrition and Aging on Eye Lens Protein and Protease Function

Mission

To determine the primary causes of eye lens cataract and to use that knowledge to extend the useful life of the lens, primarily via determining adequate levels of nutrients during various stages of life.

The laboratory pursues this mission principally using human and other mamalian lens tissue, a variety of animal models, whole lenses in culture and lens epithelial cells in culture.

Investigators

Allen Taylor, PhD Laboratory Chief/Scientist I Associate Professor, Biochemistry and Nutrition	Provides leadership to the laboratory. Coordinates human studies regarding the protective effects of ascorbate against cataractogenic changes in the lens. Grows crystals of leucine amino-peptidase. Assists in molecular biological studies of protease function and on studies regarding the ability of underfeeding to delay the onset of cataract in mice.
Ruth Lipman, PhD* Research Associate	Studies dietary restriction as a means of delaying the onset of cataractous changes in Emory and CFW mice. Performs experiments regarding the breakdown of proteins from lenses modified by glycosylation, acylation, etc.
Joseph Berger, PhD Research Associate	Studies the optimal dietary intake of ascorbate to delay UV-induced cataract-like damage to lens proteins *in vivo* and in cells in culture.
Jessica Jahngen, MS* Research Associate	Conducts research on ability of the lens to recognize and edit its proteins and ability to recognize damaged lens proteins as altered by age.

Technical Support

Cynthia Peltier, MS, Graduate Research Assistant
Dan O'Reilly, MS, Research Assistant
Mairi White, Research Assistant
Libby Jensen, Staff Assistant

Current Projects

Determination in guinea pigs of the optimal levels of dietary ascorbate for maximal protection of the lens against cataract-like changes in the lens.

Effect of dietary restriction on the onset of senile-type cataract in the Emory and CFW mouse.*

Effect of dietary antioxidant supplementation on the appearance of cataract in Emory and CFW mice.

Effect of ascorbate on light-induced aminopeptidase inactivation in whole rabbit lenses in culture.*

Effect of ascorbate supplementation on light-induced damage to proteases in cultured lens epithelial cells from beef, rabbits, and humans.*

Factors effecting initiation of proteolysis in the lens: Ubiquitinization of lens proteins and the breakdown of ubiquitin and its conjugates.*

Effect of antioxidant nutrient intervention on the functional life of enzymes in beef and rabbit lens cells in culture which catalyze the initiation of proteolysis.

Effects of nutrient status and lysosome protease activities in lens tissue or lens cultured cells from beef and rabbit.*

Roles of zinc, manganese, and magnesium in proteolysis (leucine aminopeptidase) from beef lens.*

Effect of ascorbate on light-induced protein polymerization in beef and rabbit lens cells in culture and in homogenates.

Effect of vitamin E on albuminoid formation and altered proteolytic capability in 657BL/j6 mice.

Levels of vitamins A and E in lens tissue from aging animals.

Role of nutrition in the occurence of senile lens opacities: an epidemiological and nutritional biochemistry study.

Recent Research Accomplishments

Studies on the bioavailability of ascorbate in the lens. It has been established that guinea pigs with low dietary ascorbate have proportionately lower lens ascorbate and that raising dietary ascorbate results in increased lenticular ascorbate. Lenses of animals fed elevated dietary ascorbate are better able to withstand UV-induced age-related damage.

Ascorbate protects against photooxidative damage to lens proteins and proteases. Solar simulated light (UV A and B) cause lens protein aggregation similar to that which occurs in cataracts. This follows certain protease inactivation. Ascorbate delays both processes. This implies that attenuation of the protein catabolizing machinery may be causally related to the accumulation of damaged proteins which is associated with cataract and that dietary antioxidants may offer protection against these insults.

Tocopherol and carotenes effects on lens integrity. Prior to testing the effects of tocopherol and carotenes on the stressed lens it was necessary to establish values for these nutrients. The levels of tocopherol and vitamin A have been determined in beef lenses.

The effect of development, aging and nutrition on ubiquitin dependent protein editing. Proteolysis provides a method of editing the cellular proteins and recycling the amino acids of disposable proteins. It has recently been shown that in some cells proteolysis is initiated upon conjugation with ubiquitin to the substrate protein in an ATP dependent reaction. The lens was found to contain: 1) ubiquitin, 2) enzymes capable of catalyzing the conjugation of proteins to ubiquitin, 3) endogenous ubiquitin conjugates, 4) ATP-dependency of the process and 5) an age-related impairment in the ability of lens cells to catalyze conjugation. Protein aggregates accumulate with age and are associated with cataract and are substrates for the ubiquitin proteolytic system. Thus, it is plausable that inactivation of this proteolytic machinery is related to the aggregation of proteins and appearance of cataract in aged lens tissue.

Defining the protein editing machinery of the lens during development and aging. Several new proteolytic enzymes in the human, beef and rabbit lens has been discovered. One has been partially purified. The activities of proteases which make up the lens protein editing machinery have been followed in cells from different age lenses and in cells taken from young animals but in which age was mimicked by extensive "passage".

Selected Recent Publications

Oettgen, H.C. and Taylor, A. Purification and Characterization of Leucine Aminopeptidase from Hog Lens. Analyt. Biochem 146:238-245, 1985.

Jahngen, J.H., Eisenhauer, D.A. and Taylor A. Lens Proteins are Substrates for the Reticulocyte Ubiquitin Conjugation System. Curr. Eye. Res 5:725-733, 1986.

Jahngen, J., Haas, A.L., Ciechanove, A., Blondin, J., Eisenhauer, D. and Taylor, A. The Eye Lens Has an Active ATP-dependent Ubiquitin Protein Conjugation System. J. Biol. Chem 261:13760-13767, 1986.

Blondin J., Sadowski, J., Baragi, V.J., Schwartz, E. and Taylor, A. Dietary Ascorbate Supplements Delay the Onset of Protein Aggregation and Enzyme Inactivation in UV Irradiated Quinea Pig Lenses. J. Nutrition (Submitted 1986).

Taylor, A. and Jahngen, J.H. Eye Lens Maturation and Aging are Associated with Decrements in Activity of the Ubiquitin/ATP Proteolytic Pathway in Environmental Design for Optimal Vision in the Elderly, Plenum Press, NY, Armstrong, D., ed. (In Press, 1986).

Taylor, A. Leucine Aminopeptidase Activity is Diminished in Aged Hog, Beef and Human Lens. In: Intracellular Protein Catabolism. Kharallah, E., Bond, J.S. and Bird, J.W.C., eds. Alan R. Liss, Inc., New York pp. 299-302, 1985.

Taylor, A., Surgenor, T., Thomson, D.K.R., Graham, R.J. and Oettgen, H.C. Comparison of (Concentration and Amino Acid Sequence Homology Between) Leucine Aminopeptidase in Human Lens, Beef Lens, Beef Kidney, Hog Lens, and Hog Kidney. Exp. Eye Res. 38:217-229, 1984.

Taylor, A., Volz, K., Lipscomb, W.N. and Takemoto, L. Leucine Aminopeptidase Techniques, Electron Microscopy and X-ray Diffraction. J. Biol. Chem. 259:14575-14761, 1984.

NUTRITION AND CELL PROGRAMMING LABORATORY

CRIS: New Methods for Assessing Protein Requirements

Impact of Nutrition on Cell Programming and Regulation During Aging

Mission

To better identify optimal nutrient intakes at different stages of life, new criteria for adequacy of nutrient supply based on bioregulatory mechanisms are being sought. The program focuses primarily on assessing the adequacy of protein and energy intake. Regulatory mechanisms, notably endocrine control, are studied at critical periods of life, namely pregnancy, adolescence and the aging adult. The response of endocrine factors, notably the somatomedins, to various levels of dietary protein are being tested as a criterion of nutrient adequacy.

Since growth, development and senescence occur through changes in bioregulatory mechanisms, the impact of nutritional status of those bioregulatory mechanisms during certain key periods of the life span are being explored using modern methods appropriate to gene function. Thus, the laboratory is developing appropriate techniques for exploring the aging genome and applying them to the study of long-term dietary effects on nutrient-sensititive control mechanisms of cells and tissues during the aging process.

Investigators

Hamish Munro, MD, DSc Laboratory Chief/Senior Scientist Professor, Medicine and Nutrition	Provides program planning including studies of somatomedin responses to nutritional factors throughout the life span and studies of the metabolism of protein and carbohydrate in aging by new techniques.
Mark Hellerstein, MD, PhD Scientist III	Conducts research on intermediary metabolism of dietary carbohydrate and on plasma protein synthesis in response to stress.

Technical Support

Kristin White, MS, Graduate Research Assistant

Current Projects

Somatomedin assay techniques for field studies.

Plasma somatomedin responses to adolescence of children in relation to nutritional status.

Diet-plasma somatomedin level relationships in the elderly.

Techniques for studying carbohydrate and protein metabolism in aging animals and human subjects.

Plasma glycoprotein synthesis in response to stress in senescent animals and elderly humans receiving different levels of dietary protein.

Metabolic events in galactose utilization by intact animals, using a novel technique.

Glucose tolerance and metabolism in the elderly in relation to intake of glucose and galactose.

Peroxidation of tissue components in relation to iron accumulation with aging.

Control mechanisms of the ferritin genome during aging and its relationship to iron accumulation.

Recent Research Accomplishments

Plasma somatomedin levels are sensitive to nutritional conditions. The levels of somatomedins circulating in rat plasma of mother and pups during pregnancy are reduced by protein deficiency of the dam. This has been traced to a reduced output of lactogenic hormone from the placenta. During pregnancy, placental lactogen replaces pituitary growth hormone in regulating output of somatomedins; protein deficiency impairs the capacity of the placenta to make the lactogen. Somatomedin levels can be corrected by infusing lactogen.

Normal increases in somatomedin-C in plasma during the puberty growth spurt are greatly attenuated in adolescent children with a recorded episode of severe protein-calorie malnutrition in infancy. This phenomenon may be attributed to a lack of proper hypothalamic development caused by the episode of malnutrition, with consequent lack of the normal response of growth hormone output during puberty.

A Boston elderly population shows no evidence of insufficency of protein intake. Protein intake averaged 1.05 gm protein/kg body weight in healthy, free-living subjects over 60 years. Concentrations of several blood proteins were examined as indicators of protein malnutrition. Slight reductions in their concentrations were related to age but were not due to insufficient protein intake. Measurements of upper arm muscle mass also showed no relationship between the amount of muscle and protein intake. Thus, protein malnutrition is not a likely hazard amoung the non-diseased elderly in the U.S.

New Methods for studying plasma glycoprotein synthesis by the liver. A new procedure has been developed for probing intermediates in isotopic labeling of hepatic carbohydrate metabolism by trapping hepatic glucuronic acid with acetaminophen, the resulting glucuronide being excreted in the urine. This glucuronide is in equilibrium with UDP-glucose from which it is directly derived; UDP-glucose is the source of UDP-galactose. This provides a method for comparing the galactose labeling in plasma glycoproteins with that of their precursor UDP-l-galactose, using the glucuronide label at steady-state. Using doubly-labeled [3H]/[14C]-glucose as precursor, two pools of hepatic UDP-glucose are evident, one associated with the endoplasmic reticulum and feeding into galactose and glucuronic acid and the other supplying glucose to glycogen. Using rats injected with turpentine, it has been shown by this technique that synthesis of a stress-associated glycoprotein (AGP) is greatly accelerated in the liver.

Selected Recent Publications

Aziz, N. and Munro, H.N. Both Subunits of Rat Liver Ferritin are Regulated at a Translational Level by Iron Induction. Nucl. Acids Res. 14:915,927, 1986.

Middleton, T., Herlihy, W.C., Schimmel, P.R. and Munro, H.N. Synthesis and Purification of Oligoribonucleotides Using T4 RNA Ligase and Reverse-Phase Chromatography. Analyt Biochem. 146:366-371, 1985.

Dorner, M.H., Salfeld, J., Will, H., Leibold, E.A., Vass, J.K. and Munro, H.N. Structure of Human Ferritin Light Subunit Messenger RNA: Comparison with Heavy Subunit Message and Functional Implications. Proc. Natl. Avad. Sci. USA 82:3139-3143, 1985.

Munro, H.N., Leibold, E.A., Vass, J.K., Aziz, N., Rogers, J., Murray, M. and White, K. Ferritin Gene Structure and Expression. In: Proteins of Iron Storage and Transport (G. Spik, J. Montreuil, R.R. Crichton and J. Mazurier, eds.). Elsevier Science Publishers, B.V. pp. 331-341, 1985.

Bonmford, A.B. and Munro, H.N. Transferrin and Its Receptor: Their Roles in Cell Function. Hepatology 5:870-875, 1985.

Pickering, L., Pang, H., Biemann, K., Munro, H. and Schimmel, P. Cloning and Sequence Analysis of Rabbit Brain Creatine Kinase and Comparison with its Muscle Counterpart. Proc. Natl. Acad. Sci. USA 82:2310-2314, 1985.

Hellerstein, M.K., Sasak, V., Ordovas, J. and Munro, H.N. Isolation of Alpha-1-acid Glycoprotein from Human and Rat Plasma Using HPLC. Analyt. Biochem. 146:366-371, 1985.

Guigoz, Y. and Munro, H.N. Health Maintenance and Longevity: Nutrition. In: Handbook of the Biology of Aging. Finch, C.E. and Schneider, E.L., eds., Van Nostrand, NY, pp. 909-933, 1985.

NUTRITIONAL IMMUNOLOGY AND TOXICOLOGY LABORATORY

CRIS: The Role of Nutrition and Free Radical Reactions in Age- and Drug-Associated Changes

Nutrition, Aging and Immune Response

Mission

To understand the role of nutrients and xenobiotics on free radical formation, cell surface antigenic expression, prostanoid metabolism and membrane peroxidation events during the aging process. To elucidate the impact of these phenomena on age-related changes in nutrient requirements and chronic degenerative conditions.

The laboratory pursues this mission by exploring the effects of specific nutrients including vitamins A, C, and E, selenium, iron and dietary fat on the peroxidation of cell membranes, alterations in glycan groups of cell surface glycoconjugates and prostanoid metabolism. Emphasis is placed on examining the immunological and toxicological impact of dietary antioxidants and fatty acids on human volunteers, animal models and cell cultures.

Investigators

Jeffrey Blumberg, PhD Laboratory Chief/Scientist I Associate Professor, Nutrition	Provides leadership to the laboratory and develops integrated programs, determines research direction and examines relevance of the work.
Mohsen Meydani, DVM, PhD Scientist II Assistant Professor, Nutrition	Studies the interaction of nutrients and xenobiotics on free radical formation and lipid peroxidation and their impact on the aging process.
Simin Meydani, DVM, PhD Scientist II Assistant Professor, Nutrition	Studies the effect of dietary factors on prostanoid metabolism and immune responsiveness in elderly subjects and animal models.
Ganesa Yogeeswaran, PhD* Scientist I Associate Professor, Anatomy and Cell Biology; Nutrition	Studies function of vitamins E and A on cell surface glycoconjugates especially as they relate to immune function.

Technical Support

Chi-Chih Wu, MS, Research Assistant
Patrice McDonald, MS, Research Assistant
Margo LaRocque, BS, Research Technician*
Carl Verdon, MS, Graduate Research Assistant
George Kasparyan, MS, Graduate Research Assistant*
Cindy Fulton, Secretary

Current Projects

Effect of vitamin E supplementation on immune responsiveness in healthy elderly subjects.*

Interaction between selenium and vitamin C at low and high dietary intakes in young and old men and in Cebus monkeys.*

Effect of dietary vitamin E and selenium on in vitro lipid peroxidation in tissues from aging F344 rats.

Effect of dietary vitamin E on glutathione supplementation on the immune responsiveness of aged C57BL/6NIA mice.*

Characterization of alpha-tocopherol transfer protein in F344 rats: its functional, biophysical and biochemical alteration in aging and vitamin E deficiency.

Role of body iron stores and the effects of chronic dietary iron loading on lipid peroxidation and immune function in aging F344 rats.

Protective effects of natural and synthetic antioxidants on retinal phototoxicity in aging Long-Evans rats.

Relationship between dietary fats, vitamin E, and selenium in immune responsiveness and autocoid formation in young and aged C57BL/6NIA mice.

Effect of dietary fats and vitamin E on age-related and casein-induced amyloidosis in animal models.*

Relationship between vitamin B6 and immune response in elderly subjects.

Effect of vitamin E supplementation on exercise-induced lipid peroxidation in old and young men.*

Effect of vitamin E-rich lipoproteins on cytotoxicity and prostaglandin synthesis in endothelial cell culture.

Effect of age and sex on postprandial plasma and lipoprotein concentration of tocopherols in healthy human subjects.

Role of carbohydrate determinants of glycoproteins and glycolipids as potential target structures in mouse lymphoma cells and characterization of the target receptors for natural killer cells.*

Requirements for vitamin A in normal embryo differentiation and the role of glycoconjugates as mediators and markers of differentiation using an F9 mouse embryonal carcinoma model.*

Regulation of glycoconjugate metabolism by vitamin E: effects on microtubules and glutathione systems and consequences for antigenicity and tumorigenicity using PMN and K3T3 reticulum sarcoma cells.*

Recent Research Accomplishments

Vitamin E and glutathione have immunostimulant properties in senescent mice. Decreased prostaglandin E2 (PGE2) synthesis in vitamin E supplemented mice enhanced in vivo and in vitro immune responsiveness. Spleens from aged mice produced significantly more PGE2 than young mice which may contribute to their decreased immune responsiveness. Vitamin E supplementation also decreased the development of spontaneous kidney amyloidosis in aged mice. Dietary supplementation with glutathione enhanced mitogenic responses and delayed type hypersensitivity in old mice. Low spleen glutathione levels characteristic of old mice were increased by glutathione supplementation. Thus, it appears that improving vitamin E or glutathione status enhances immune responsiveness in aged mice.

Dietary fish oil effects vitamin E status and prostanoid metabolism. Mice fed fish oil have significantly less plasma, liver and kidney tocopherol levels than mice fed corn oil or coconut oil. The adverse change in tocopherol status induced by fish oil feeding might interfere with some of the beneficial effects attributed to increased marine oil consumption. Dietary fish oil dramatically decreased formation of PGE2 by splenocyte culture which was associated with a modest increase in lymphocyte proliferation. The lack of a strong correlation between lower PGE2 synthesis in fish oil fed mice and enhancement of lymphocyte proliferation might be due to a compromised vitamin E status. In lungs, fish oil fed mice had lower thromboxane B2 (TXB2) and prostacycline (PGI2) levels with an overall increase in the TXB2/PGI2 ratio, especially in aged mice. Supplementation of fish oil diets with tocopherol decreased the TXB2/PGI2 ratio. Diets containing fish oil significantly decrease the severity of azocasein-induced amyloidosis. This effect of fish oil is associated with a change in macrophage PGI2 production.

Effect of chronic dietary iron loading on in vivo lipid peroxidation and in vitro immune responsiveness in F344 rats. Iron loading (2.5% diet) decreased food intake in young and aged rats and increased lipid peroxidation and total prostaglandin synthesis. In vitro spleen PGE2 synthesis was reduced and was associated with enhanced lymphocyte proliferation. No significant effect on interleukin 2 production was observed. Iron loaded rats had lower liver copper concentrations. The capacity of ferritin synthesis to respond to high dietary iron appears unimpaired during aging.

Effect of vitamin E on cell surface glycoconjugate metabolism. Terminal glycan groups in the glycoproteins and glycolipids of natural killer (NK) target cells were inversely correlated with the susceptibility of target cells to NK-cell mediated lysis. Vitamin E administered to cultured transformed reticulum cells causes a generalized decrease in the sialylation and cell surface expression of glycoproteins and glycolipids in a dose-dependent manner. The effect on microtubule assembly may be mediated via sialyl transferase or the antioxidant property of vitamin E.

Postprandial alterations in lipoprotein alpha-tocopherol. Postprandial alpha-tocopherol was found to enter plasma in chylomicrons and very low density lipoprotein and then transfer to low density lipoprotein and high density lipoprotein with further exchange between lipoproteins. Vitamin E absorption peaked 6 hours postprandially. Gamma-tocopherol is absorbed and incorporated into the lipoprotein particles at a slower rate than alpha-tocopherol.

Rates of lipid peroxidation reactions and prostanoid metabolism are strongly influenced by dietary vitamin E and/or selenium. Dietary vitamin E and selenium were found to offer protection against spontaneous, nutrient (iron, vitamin C) and xenobiotic-induced free radical reactions in liver and brain of young and senescent rats. Differential susceptibility of brain regions (cerebellum > cerebral cortex > midbrain > brain stem) to lipid peroxidation was correlated with alpha-tocopherol concentration; the data indicate some brain regions possess a higher dietary requirement for vitamin E than current allowances. PGE2 synthesis in brain was found to decrease with age and was associated with changes in alpha-tocopherol levels. Ethoxquine, a synthetic antioxidant added to diet, was more potent in reducing PGE2 synthesis in brain regions than vitamin E. Mice fed fish oil supplemented with vitamin E had significantly lower PGE2 synthesis in brain regions than those fed with coconut oil. Dietary vitamin E and selenium were also found to alter platelet aggregability through effects on platelet TXA2 and aortic PGI2.

Selected Recent Publications

Blumberg, J.B. and Meydani, S.N. "Role of Dietary Antioxidants in Aging." In: Bristol-Myers Nutrition Symposia: Nutrition and Aging, Academic Press, NY 5:85-97, 1986.

Meydani, S.N., Cathcart, E.S., Hopkins, R.E., Meydani, M., Hayes, K.C. and Blumberg, J.B. Antioxidants in Experimental Amyloidosis of Young and Old Mice. In: Amyloidosis (Proceedings of the IV International Congress). G.G. Glenner, E.P. Asserman, E. Benditt, E. Calkins, A.S. Cohen, D. Zucker-Franklin, eds. Plenum Press, NY, pp. 683-692, 1986.

Meydani, S.N., Meydani, M., Verdon, C.P. and Blumberg, J.B. Vitamin E Supplementation Suppresses Prostaglandin E_2 Synthesis and Enhances the Immune Response of Aged Mice. Mechanisms of Ageing and Development 34:191-201, 1986

Meydani, M., Verdon, C. and Blumberg, J.B. Effect of Dietary Vitamin E, Selenium and Age on Lipid Peroxidation Events in Rat Cerebrum. Nutrition Research 5:1227-1236, 1985.

Blumberg, J.B. Drug Metabolism in the Aged. Drug-Nutrient Interactions 4:99-106, 1985.

Meydani, M., Meydani, S., Macauley, J.B. and Blumberg, J.B. Influence of Dietary Vitamin E and Selenium on the Ex-vivo Synthesis of Prostaglandin E_2 in Brain Regions of Young and Old Rats. Prostaglandins and Leukotrienes in Medicine 18:337-346, 1985.

Meydani, M., Blumberg, J.B. and Hayes, K.C. Dietary Fat Unsaturation Enhances Drug Metabolism in Cebus but not in Squirrel Monkeys. Journal of Nutrition 115:573-578, 1985.

Yogeeswaren, G. and Mbawuike, I.N. Altered Metabolism and Cell Surface Expression of Glycosphingolipids Caused by Vitamin E in Cultured Murine (K3T3) Reticulum Sarcoma Cells. Lipids 21:643-647, 1986.

PHYSIOLOGY LABORATORY

CRIS: Relationships Between Aging, Functional Capacity, Body Composition and Substrate Metabolism and Needs

Mission

To explore the interaction between nutrition, exercise and aging and to understand how physical activity affects nutrient requirements and functional capacity in the elderly. The extent to which aging alters the adaptive responses to increased physical activity is largely unknown, particularly in the effects on protein metabolism. The laboratory is focusing on the metabolism and requirements for some of the macronutrients and how they change with age and activity.

The laboratory is making use of stable isotope probes and the euglycemic glucose clamp technique to establish how energy expenditure, body composition and the turnover of whole body nitrogen and glucose vary in the normal population with increasing age, particularly with regard to changes in amount of physical activity. Through the use of these techniques, it can be established how these changes affect substrate requirements.

Investigators

William Evans, PhD Laboratory Chief/Scientist II Assistant Professor, Nutrition	Directs laboratory research program. Performs muscle biopsies for biochemical, histochemical and ultrastructural examination.
Carol Meredith, PhD Scientist III Lecturer, Nutrition	Conducts research on effects of age and activity on protein metabolism using nitrogen balance techniques and stable isotope probes.
Russ Hauser, MD* Research Associate	Provides medical support for all Physiology studies. Conducts research on effects of physical rehabilitation and strength training on muscle metabolism in the elderly. Establishes normal values for muscle strength in elderly.

Technical Support

Virginia Hughes, MS, Laboratory Manager
Elizabeth Fisher, MS, Research Assistant*
Kevin O'Reilly, MS, Research Assistant*
Roger Fielding, MA, Laboratory Technician
Miriam Nelson, MS, Graduate Research Assistant
Katherine Yannotti, Secretary

Current Projects

Effects of aerobic training on body composition, physical fitness, and amino acid metabolism in young and old men and women.

Effects of strengthening exercise on the protein requirements, whole body protein turnover and albumin synthesis rate of middle-aged and young men.

Evolution of muscle damage and protein turnover following eccentric exercise in untrained young and old men.

Effects of dietary calcium and weight bearing exercise on calcium metabolism and bone density in post-menopausal women.*

Effects of vitamin E supplementation on muscle membrane damage and acute phase response after eccentric exercise in elderly and young men.*

Reproducibility of graded maximal exercise tests in elderly women.

Effects of age on creatinine clearance, body composition and strength.

Effects of age, diet and training on glucose metabolism.*

Recent Research Accomplishments

Protein requirements are higher than the current USRDA for physically active older men. Dietary requirements for protein intake have not yet been determined for middle-aged men nor for subjects who habitually engage in endurance exercise. Studying dietary protein intakes between 0.59 and 1.18 g/kg.day, mean protein requirement (using nitrogen balance) for this active population would be 0.92 g/kg.day indicating an RDA of 1.39 g/kg.day of quality protein, a value considerably higher than the current standard of 0.8 g protein/kg.day.

Exercise induced muscle damage is associated with alterations in plasma interleukin-1 (IL-1) levels and increased protein breakdown. Certain forms of exercise are associated with significant amounts of skeletal muscle damage. One bout of high intensity eccentric exercise caused a dramatic increase in serum creatine kinase activity which was associated with changes in plasma IL-1 levels, a substance thought to mediate changes in protein metabolism. Muscle protein breakdown was significantly elevated ten days following exercise.

Amenorrheic athletes have significantly lower bone mineral density (BMD) of the lumbar spine than eumenorrheic athletes. Amenorrheic athletes provide a model to study the effects of low estrogen on bone health. Young women who have become amenorrheic as a result of exercise have hormone profiles similar to postmenopausal women. BMD of the lumbar spine was significantly related to plasma estrogen levels. The amenorrheic runners had a reported energy intake significantly lower than their eumenorrheic counterparts which may be associated with the development of amenorrhea.

Older men and women respond to aerobic exercise training with significant changes in skeletal muscle metabolism. After a 12 week aerobic conditioning program elderly men and women showed a greater relative increase in functional capacity than did a group of young men and women performing similar exercise. In addition, muscle biopsies revealed significant increases in oxidative capacity and muscle glycogen reserves in the older subjects that were not apparent in the young group.

Exercise increases muscle size and strength in elderly men. Twelve weeks of high resistance strength training caused a significant increase in muscle strength and muscle size (measured by CT scan and muscle biopsy). In addition, the training caused a significant reduction in fasting plasma insulin levels. Thus, the skeletal muscle of the elderly is quite responsive to strength training.

Exercise that causes skeletal muscle damage is associated with a diminished ability to resynthesize glycogen. Certain forms of exercise are associated with significant amounts of skeletal muscle damage that is long lasting. This damage is associated with a greatly diminished ability to resynthesize muscle glycogen as muscle biopsies taken ten days after exercise revealed very low glycogen levels. This occurred despite the fact that the subjects were inactive and maintained a high carbohydrate diet for the entire period.

Regularly performed treadmill exercise can increase the capacity to mobilize and oxidize fat in old rats. Aging is associated with a diminished capacity for fat catabolism. This age-related change was reversed by exercise training and the magnitude of these changes was directly related to the relative stress of the exercise to the animal as measured by adrenaline levels.

Middle-aged active men have a reduced muscle mass and higher rate of muscle protein turnover than do young active men. While rates of whole body protein turnover, synthesis and breakdown were not different between young and middle-aged men, measurement of the urinary 3-methylhistidine/creatinine ratio revealed a greater contribution of muscle protein turnover to whole body protein turnover in middle-aged when compared to young men.

Selected Recent Publications

Fielding, R.A., Evans, W.J., Hughes, V.A., Moldawer, L.L. and Bristian, B.R. The Effects of High Intensity Exercise on Muscle and Plasma Levels of Alpha-ketoiscaproic Acid, Eur. J. Appl. Physiol. 55:482-485, 1986.

Nelson, M.E., Fisher, E.C., Catsos, P.D., Meredith, C.N., Turksoy, R.N. and Evans, W.J. Diet and Bone Health in Amenorrheic Runners. Am. J. Clin. Nutr. 43:910-916, 1986.

Evans, W.J., Meredith, C.N., Cannon, J.G., Dinarello, C.A., Frontera, W.R., Hughes, V.A., Jones, B. H. and Knuttgen, H.G. Metabolic Changes Following Eccentric Exercise in Trained and Untrained Men. J. Appl. Physiol. 61(5), 1986).

Fisher, E.C., Nelson, M.E., Frontera, W.R., Turksoy, R.N. and Evans, W.J. Bone Mineral Content, Gonadotropic and Estrogenic Hormone Levels in Amenorrheic Runners. J. Clin. Endo. & Metab. 62:1232-1236, 1986.

Cannon, J.G., Dinarello, C.A., Evans, W.J., Hughes, V.A. and Meredith, C.N. The Initiation of an Acute Phase Response to Exercise: Mechanisms Contributing to Increased Interleukin/Production. J. Appl. Physiol. 61(5), 1986.

Evans, W.J. and Hughes, V.A. Dietary Carbohydrates and Endurance Exercise. Am. J. Clin. Nutr. 41:1146-1154, 1985.

Warhol, M.J., Siegel, A.J., Evans, W.J., and Silverman, L.M. Skeletal Muscle Injury and Repair in Marathon Runners After Competition. Am. J. Pathol. 118:331-339, 1985.

PROGRAM IN NUTRITIONAL EPIDEMIOLOGY

CRIS: Nutrition, Epidemiology and Aging

Mission

To identify the determinants of nutritional status in the elderly and to relate nutritional status to health and well-being. The focus of these studies is on the interrelationships among: age-associated changes in energy and nutrient intake; constitutional and environmental determinants of food choices; use of vitamin-mineral supplements and medications; biochemical markers of nutrient status; anthropometric, blood pressure, and other indicators of health status such as age-related changes such as senile lens opacification.

Investigators

Stuart Hartz, ScD Program Chief/Scientist I Associate Professor, Community Health and Nutrition	Provides leadership to the program. Designs study protocols and data analysis strategies to investigate project hypotheses. Conducts research on drug-nutrient interactions in the elderly and explores the nutritional epidemiology of eye disease.
Robert McGandy, MD Senior Scientist Professor, Nutrition	Conducts research on the nutritional status of the elderly.
Paul Jacques, MS Research Associate	Conducts research on the relationship between diet and cataractogenesis and between vitamin C and plasma lipoprotein regulation.

Technical Support

Constance Otradovec, MA, Project Manager
Sandra Sulsky, BA, Research Study Coordinator*
Dee Garretson, BA, Research Assistant

Current Projects

Studies on the role of nutrition in the occurrence of senile lens opacities in 150 Boston residents.*

A comprehensive nutritional status survey of 1,000 free-living and institutionalized Boston elderly persons including anthropometric, biochemical and clinical assessments.

A study of the use and effect of nutrient supplements on nutritional status in 750 Boston elderly persons.

A study of the relationship of drug-nutrient interactions and nutritional status in 1,000 Boston elderly persons.

Recent Research Accomplishments

Nutritional status of 700 non-institutionalized elderly (ages 60-98) in the Boston area. Between ages 60-98 years, energy intake significantly decreases in males but not in females. Considering nutrients from diet alone, more than 15% of these subjects have intakes less than two-thirds the Recommended Dietary Allowance (RDA) for vitamins A, D, B_6, B_{12}, folate, calcium and zinc. Among the factors associated with significantly reduced dietary nutrient quality are low family income, low educational attainment and (particularly for males) use of dentures. Among the factors associated with supplement use are high educational attainment and persons reporting problems chewing food. Over 10% of the females have combined nutrient intakes (diet plus supplement) that exceed 10 times the RDA for vitamins B_1, B_2, B_6, B_{12}, D and E. Among males, comparably high intakes are observed for vitamins B_1, B_6, C, E, and iron.

Relationships of blood levels of vitamins C and E with lipoproteins in non-institutionalized elderly. Even beyond age 70, both high density lipoprotein cholesterol (HDL-C) and, less strongly, low density lipoprotein cholesterol (LDL-C) are reported to be predictors of atherosclerotic vascular disease. In this survey, plasma vitamin E levels were positively associated with LDL-C and with HDL-C. Plasma vitamin C levels were positively associated with HDL-C levels among both sexes, a correlation which reached statistical significance only among women.

Nutritional status of an elderly institutionalized population. Nutrient intakes, biochemical assessments, anthropometrics, and social, demographic and health-data were collected on 260 institutionalized males and females aged 60 years and older from 15 institutions, included 98 mentally incompetent residents of a state hospital. Overall, dietary intakes were adequate when compared to two-thirds of the 1980 RDA's. Only intakes of calories (males), vitamins A (males), B6, and D, folate and zinc appeared to be potential problems. However, biochemical assessment of fasting samples revealed low values of transferrin, prealbumin, albumin and retinol binding protein. Anemia, as defined by low hemoglobin and hematocrit values, appeared to be more prevalent in males than in females, although young adult standards may be too high for the elderly.

Nutritient supplement use by healthy elderly. As part of a nutritional status survey of 691 free-living men and women aged 60 years and older, supplement use was reported by 45% of the males and 55% of the females. Supplement use was more prevalent in females than males at each age decade. Vitamins C and E were most commonly used supplements. The percentage of dietary intakes falling below 2/3 RDA was comparable for users and non-users of supplements. Use of supplements markedly decreased the proportion of subjects with inadequate nutrient intake (using a 2/3 RDA criterion), particularly for vitamins B6, B12 and D, folic acid and calcium. However, for both males and females, potentially excessive intake levels (ten times the RDA) of thiamin, vitamin A and vitamin E supplementation were observed.

Selected Recent Publications

McGandy, R.B., Russell, R.M., Hartz, S.C., Jacob, R.A., Tannenbaum, S., Peters, H., Sahyoun, N. and Otradovec, C.L. Nutritional Status Survey of Healthy Noninstitutionalized Elderly: Energy and Nutrient Intakes from Three-Day Diet Records and Nutrient Supplements. Nutrition Research 6:785-798, 1986.

Hartz, S.C. and Blumberg, J. Use of Vitamin and Mineral Supplements by the Elderly. Clinical Nutrition 5:130-136, 1986.

McGandy, R.B. Nutrition. In: The Teaching Nursing Home. Schneider, Ed. Raven Press, pp. 207-212, 1985.

McGandy, R.B. Nutrition. In: Practical Geriatric Medicine. Exton-Smith and Webster, eds. Churchill Livingstone, pp. 43-50, 1985.

Papas, A., Herman J., Palmer, C., Rounds, M., Russell, R., McGandy, R., Hartz S., Jacob R. and Feldman R. Oral Health Status of the Elderly with Dieary and Nutritional Considerations. Gerodontology 3: 147-155, 1984.

Adelman, M.O., Dwyer, J.T., Woods, M., Bohn E. and Otradovec, C.L. Computerized Dietary Analysis Systems: A Comparative View. J Am Diet Assoc: 83:421-429, 1983.

Jacques, P.F., Hartz, S.C., McGandy, R.B., Jacob, R.A. and Russell, R. Ascorbic Acid, HDL, and Total Plasma Cholesterol in the Elderly. Amer J Clin Nutr (In Press, 1986).

VITAMIN D AND BONE METABOLISM LABORATORY

CRIS: Micronutrient Requirements of the Elderly

Mission

To determine whether aging adversely affects the capacity of human skin to produce vitamin D3 and the ability of the gastrointestinal tract to absorb dietary vitamin D3. To develop guidelines for the elderly regarding skin exposure to sunshine as a means of providing them with adequate vitamin D nutrition at times when dietary sources are insufficient.

The laboratory pursues this mission by: (a) determining the effect of aging on the concentrations of provitamin D3 in human skin, (b) exposing human skin from individuals to simulated sunlight and determining how aging affects the photosynthesis of provitamin D3, (c) evaluating the ability of cultured human keratinocytes to synthesize provitamin D3, and (d) determining if aging affects the blood concentrations of vitamin D after oral administration of vitamin D. It is becoming clear that a significant number of the elderly population are not receiving adequate vitamin D nutrition from dietary and solar sources. Results from these research activities should provide a better understanding of how to provide adequate vitamin D nutrition for the elderly.

Investigators

Michael Holick, PhD, MD* Laboratory Chief/Senior Scientist Professor, Physiology and Nutrition	Provides leadership to the laboratory. Develops new chromatographic techniques for vitamin D and directs all clinical research activities.
Sally Ann Holick, PhD* Scientist III	Develops synthetic methods of making vitamin D water soluble and studies the chemistry of vitamin D.
Rahul Ray, PhD* Scientist III	Synthesizes analogs of vitamin D and its metabolites. Develops methods related to 1,25-(OH)2-D3 receptor.

Technical Support

Nancy Hanafin, BA, Laboratory Supervisor*
Ellen Smith, PhD, Research Assistant*
Ann Webb, PhD, Research Assistant*
Julia McLaughlin, MS, Research Assistant*
Lynn Donovan, BS, Research Assistant*
Louise Palmer, BS, Research Assistant*
Kelly Scott Persons, BS, Research Assistant*
Louise Fred, Medical Editor
Catherine St. Claire, B.A., Staff Assistant

Current Projects

Determination of the effect of aging on cutaneous provitamin D3 concentrations in elderly humans.

Evaluation of aging on vitamin D nutrition after whole-body exposure to ultraviolet radiation.

Physiologic role of 1,25-dihydroxyvitamin D3 on epidermal proliferation and maturation.

Synthesis of analogs of vitamin D and its metabolites and their biologic evaluation.

The use of 1,25-(OH)2-D3 and its analogs for the prevention and treatment of age-related skin disorders.

Isolation and identification of new forms of vitamin D from phytoplankton and other marine species.

The physiologic role of exposure to sunlight for vitamin D nutrition.

Recent Research Accomplishments

Aging decreases the capacity of human skin to produce vitamin D3. Current results suggest that there is an inverse relationship between the concentrations of provitamin D3 in the epidermis with age. These data suggest that aging decreases the capacity of human skin to produce vitamin D.

Identification of a new cutaneous provitamin D. It has been assumed that the skin produces only one vitamin D, vitamin D3, as a result of exposure to sunlight. An analysis of rat and human skin has revealed that there is another provitamin D in the epidermis that has been identified as delta^{24}-provitamin D3.

Synthesis of a photoaffinity derivative of 1,25-dihydroxyvitamin D3. 1,25-dihydroxyvitamin D3 is essential for the maintenance of a healthy skeleton. This hormone must be recognized by a specific cytosolic and nuclear receptor for it to carry out its physiologic actions. Little is known about the binding site(s) of these receptors for 1,25-(OH)2-D3 and what effect aging has on this physiologic process. To address these issues, a photoaffinity derivative of 1,25-(OH)2-D3 has been synthesized that is recognized by the receptors and can be covalently linked to them.

The role of 1,25-dihydroxyvitamin D3 on the differentiation of cultured human keratinocytes. Cultured human keratinocytes have been found to possess cytosolic and nuclear receptors for 1,25-(OH)2-D3. When cultured keratinocytes are exposed to 1,25-(OH)2-D3 this hormone stimulates differentiation. Results suggest that cultured skin cells from patients with disorders of epidermal differentiation are less responsive to 1,25-(OH)2-D3.

The effect of season on the cutaneous production and degradation of vitamin D3. A model has been developed to determine how effective exposure to sunlight during different times of the day, month and year is for cutaneous vitamin D3 production and degradation. Vitamin D3 appears exquisitely sensitive to sunlight and photolyzes to suprasterols I and II. Analysis of the conversion of provitamin D3 to previtamin D3 in Boston revealed that this reaction occurs only during the months of March through October. The data strongly suggest that exposure to sunlight between the months of November and March will not result in the cutaneous production of vitamin D3.

Selected Recent Publications

Holick, M.F. The Photobiology of Vitamin D and its Consequences for Humans. Ann New York Acad of Sci 453:1-13, 1985

Ray, R., Holick, S.A. and Holick, M.F. Synthesis of Photoaffinity-Labelled Analog of 1,25-dihydroxyvitamin D_3. J Chem Soc (London), Chem Commun 11:702-703, 1985.

Ray, R., Rose, S., Holick, S.A. and Holick, M.F. Evaluation of Photoabile Derivative of 1,25-dihydroxyvitamin D_3 as a Photoaffinity Probe for 1,25-dehydroxyvitamin D_3 Receptor in Chick Intestinal Cytosol. Biochem Biophys Res Commun 132:198-203, 1985.

Holick, S.A., St. Lezin, M., Young, D., Malaikal, S. and Holick, M.F. Isolation and Identification of 24-Dehydroprovitamin D_3 and its Photolysis to 24-Dehydroprovitamin D_3 in Mammalian Skin. J Biol Chem 260:12181-12184, 1985.

Smith, E., Walworth, N.C., and Holick, M.F. Effect of 1,25-dihydroxyvitamin D_3 on the Morphological and Biochemical Differentiation of Cultured Human Epidermal Keratinocytes Grown Under Serum-Free Conditions. J Invest Dermatol 86:709-714, 1986.

Holick, M.F. Vitamin D requirements for the elderly. Clin Nutr 5:121-129, 1986.

Holick, M.F. Role of Nutrition and Endocrine Status on Metabolic Bone Disease in the Aged. In: Migaki G. (ed). Nutritional Diseases: Research Directions in Comparative Pathobiology (Proceedings, Symposium, Nov. 1985), in press.

Lo, W., Paris, P. and Holick, M.F. Indian and Pakistani Immigrants Have the Same Capacity as Caucasians to Produce Vitamin D in response to ultraviolet irradiation. Am J Clin Nutr Nov. 1986, in press.

VITAMIN K LABORATORY

CRIS: Micronutrient Requirements of the Elderly

Mission

To develop methods for the biochemical and functional assessment of vitamin K in the elderly and to determine the nutritional sources, bioavailability and requirements of vitamin K in this population. The results obtained from these studies are being contrasted and compared to clinical and biochemical data generated from a young adult reference population. The laboratory is also focusing on changes that occur in the metabolism and function of vitamin K with aging and how these changes impact upon the clinical and nutritional status of the elderly. Ectopic calcification (vascular system,) calcium homeostasis (bone) and coagulation disorders (thrombosis) are processes that are dependent upon vitamin K and are being examined in both human and animal models.

Investigators

James Sadowski, PhD Laboratory Chief/Scientist II Assistant Professor, Nutrition	Provides leadership to the laboratory. Directs and assists in the development of the biochemical assays required for the assessment of vitamin K status. Directs all human and animal studies.
Yacoob Haroon, Ph.D. Scientist III	Conducts investigations into the requirements and metabolism of vitamin K in humans by developing analytical procedures for the assessment of vitamin K status.

Technical Staff

Constance Ganter, BA, Research Assistant
Kenneth Davidson, MS, Research Assistant
Elizabeth Cochary, MS, Graduate Research Assistant
Phyllis Allen, Secretary

Current Projects

Vitamin K requirements of aging human populations.

Vitamin K metabolism and function in aging Sprague-Dawley rats.

The cofactor requirements for the coumarin-sensitive enzymes in the vitamin K redox cycle using Sprague-Dawley rats.

The effects of gluthatione depletion and repletion on the in vivo and in vitro metabolism and function of vitamin K in Sprague-Dawley rats.

Urinary excretion of gamma-carboxyglutamic acid in humans: development and evaluation of an automated assay.

Development of an immunochemical assay for the determination of vitamin K.

Effects of aging on the absorption, transport and clearance of vitamin K in humans.

Bioavailability of vitamin K in aging human populations: effects of cooking and fat content on the test meal.

Determination of vitamin K epoxide in fasting human plasma.

Recent Research Accomplishments

Elevated levels of abnormal prothrombin may indicate vitamin K deficiency. Preliminary evidence indicates that a significant proportion of free-living elderly possess elevated levels of abnormal prothrombin. Abnormal prothrombin is formed when the specific glutamic acid residues in the amino terminal portion of the molecule are not carboxylated to gamma-carboxyglutamic acid residues. Since the carboxylation reaction requires vitamin K, the elderly subjects with elevated levels of abnormal prothrombin may be suffering from a subclinical vitamin K deficiency or their ability to metabolize the vitamin may be altered.

Development of a new HPLC assay for the determination of vitamin K in plasma. A new assay for the direct determination of vitamin K in plasma samples has been developed and validated. The vitamin is extracted from plasma by liquid-liquid extraction partition and subjected to solid phase extraction on silica gel. The vitamin K fraction from the solid phase extraction is chemically reduced and subjected to a final liquid-liquid partition prior to reverse-phase chromatography on silica-ODS. Vitamin K is detected fluorometrically after post-column zinc catalyzed conversion of the vitamin to its hydroquinone. Levels of vitamin K as low as 25 picograms/ml of plasma have been measured. With the addition of another solid phase extraction in place of the reductive extraction, vitamin K epoxide can be determined.

Vitamin K concentrations in human plasma. Fasting plasma vitamin K concentrations were determined in young and elderly reference groups by the HPLC method described above. The range for plasma vitamin K in over 340 subjects was 0.05 to 3.02 ng/ml with a mean value of 0.48 ng/ml. Greater than 95% of the subjects ranged between 0.13 and 1.30 ng/ml. Although the mean value for the younger group was lower than the elderly the differences were not statistically significant. However, when age and sex are considered, the young females were significantly different from the elderly females and elderly males. The significance is greatest when younger females are compared to the rest of the population. Thus, as humans age their fasting plasma vitamin K level gradually increases until age 60 and then decreases with the most significant changes occurring in women.

Development of an HPLC assay for the determination of gamma-carboxyglutamic acid (GLA) in urine. An automated HPLC assay for the determination of vitamin K in urine has been developed and is being evaluated for use as a method for determining vitamin K status. The urinary GLA is determined by reverse phase HPLC with fluorescence detection after dilution and automatic "on line" conversion of the GLA to its fluorescent ortho-phthalaldehyde derivative. This assay may prove useful for detecting the onset of vitamin K deficiency.

Selected Recent Publications

Bankson, D.D., Russell, R.M. and Sadowski, JA. Determination of Retinyl Esters and Retinol in Serum by Normal-Phase High-Performance Liquid Chromtography (HPLC): Method and Applications. Clinical Chemistry 32:35-40, 1986.

Haroon, Y., Bacon, D.S. and Sadowski, J.A. Chemical Reduction System for the Detection of Phylloquinone (vitamin K_1) and Menaquinones (vitamin K_2). Journal of Chromatography (In Press), 1986.

Haroon, Y., Bacon D.S. and Sadowski, J.A. Liquid Chromatographic Determination of Vitamin K_1 in Plasma with Fluorometric Detection. Clinical Chemistry (In Press), 1986.

Bacon, D.S., Haroon, Y. and Sadowski, J.A. Determination of Plasma Vitamin K Levels by Liquid Chromatography Using Post-Column Reduction and Fluorometric Detection. Fed Proc 45:842, 1986. (abs.)

Haroon, Y. and Sadowski, J.A. Automated Assay for Gamma-Carboxyglutamic Acid by Pre-Column Derivatization and Reversed-Phase Liquid Chromatography. Fed Proc 45:842, 1986. (abs.)

Cochary, E., Gershoff, S. and Sadowski, J.A. Effect of Vitamin B_6-Devoid Diet on Plasma and Tissue Pyridoxal-5-Phosphate (PLP) and Erythrocyte Aspartate Aminotransferase Acivity Coefficient (EGOT A.C.) in Rats of Different Ages. Fed Proc 45:823, 1986. (abs.)

Haroon, Y., Bacon, D.S. and Sadowski, J.A. Electrocatalytic Reduction of Quinonoid Compounds on Porous Graphite Electrodes, Presented May, 1986 HPLC Meeting, San Francisco, CA.